Sudan University of Science and Technology

College of Graduate Studies

Characterization and Study of Functional Properties of *Acacia nilotica* var. *adstringens* GUM

توصيف ودراسة الخواص الوظيفيه لصمغ السنط صنف أبوعريضه

A Thesis Submitted in Fulfillment of the Requirements for a Degree of

Doctor of Philosophy in Chemistry

By

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إستهلال

بسم الله الرحمن الرحيم

قال تعالى :

(هُوَ الَّذي أَنزَلَ مِنَ السَّماءِ ماءً لَكُم مِنهُ شَرابٌ وَمِنهُ شَجَرٌ فيهِ تُسيمونَ ﴿١٠﴾ يُنبِتُ لَكُم بِهِ الزَّرعَ وَالزَّيتونَ وَالنَّخيلَ وَالأَعنابَ وَمِن كُلِّ الثَّمَراتِ إِنَّ في ذلِكَ لَآيَةً لِقَومٍ يَتَفَكَّرونَ﴿١١﴾)

صدق الله العظيم

سورة النحل الآيات (١٠-١١)

DEDICATION

To my

Parents,

Husband, Daughters,

Brothers and sisters

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ABSTRACT

The objective of this study was to investigate parameters in *Acacia nilotica* var. *adstringens* gum, which is available in Sudan.

In total there were 45 samples of authentic *Acacia nilotica* var. *adstringens* gum were collected from North Kordofan state west of Sudan during season 2016-2017.

Physicochemical methods were undertaken to characterize the gum and to study its functional properties. Parameters such as moisture and ash contents, pH, specific optical rotation, intrinsic viscosity, nitrogen and protein content, acid equivalent weight, total uronic acid, tannin content and calorific value were determined.

The results show that the mean of moisture and ash content 11.01% and 2.06%, pH 5.06, specific optical rotation +96.4, intrinsic viscosity 11.06 cm^3g^{-1}, nitrogen content 0.06%, protein content 0.44%, acid equivalent weight 1869, total uronic acid 10.40% , tannin content 0.04% and calorific value 4.064 Kcal/g.

Acid hydrolysis of *Acacia nilotica* var. *adstringens* gum followed by (HPLC) measurements revealed that sugar content is arabinose 52%, galactose 24% and traces of rhamnose (< 1).

Cationic composition showed that calcium has the highest value among the cations studied ($3x10^{-1}$ %), followed by magnesium ($5x10^{-2}$ %), potassium, ($4.5x10^{-2}$ %), sodium ($4x10^{-2}$ %), iron ($5.8x10^{-3}$%), strontium ($4\ x10^{-3}$%), manganese ($3x10^{-3}$ %), tin ($2.4x10^{-3}$%) , zinc ($2x10^{-3}$ %), copper ($5.4x10^{-4}$%) and nickel ($9x10^{-5}$ %) respectively.

Molecular weight distribution was determined by fractionation of the gum using gel permeation chromatography. The results showed t hat three main components designated arabinogalactan protein (AGP), arabinogalactan (AG) and glycoprotein (GP) were resolved.

The molecular weight of the sample was estimated from light scattering measurement using GPC-MALLS technique. The value of M_w was found to be $2.54x10^6$ Da. The radius of gyration was found to have an average of 36.6 nm.

Emulsification studies of *Acacia nilotica* var. *adstringens* gum showed that the gum has the same emulsifying stability compared to *A. nilotica* var. *nilotica* and *A. nilotica* var. *tomentosa*.

Rheological study of *Acacia nilotica* var. *adstringens* gum show that the flow behaviour is shear thinning under low shear rate and behave as a Newtonian fluid at high shear rate. The results of oscillatory test of gum solutions revealed a typical liquid-like behaviour.

المستخلص

الهدف من هذه الدراسه هو دراسة بعض الخصائص الفيزيائيه والكيميائيه لصمغ السنط (صنف أبوعريضه) فى السودان.

تم جمع خمس وأربعين عينة من صمغ السنط (أكيشيا نايلوتيكا صنف أبوعريضه) من خمسة عشر شجرة مختلفه من ولاية شمال كردفان خلال موسمى 2017-2016 . تم توصيف صمغ السنط (أكيشيا نايلوتيكا صنف أبوعريضه) باستخدام الطرق الفيزوكيميائية حيث تم تحديد المعايير مثل محتوى الرطوبة، محتوى الرماد، قيمة الأس الهيدروجيني، الدوران الضوئي النوعي، اللزوجة الضمنية، محتوى النيتروجين و البروتين، الوزن المكافئ الحمضي و حمض اليورنيك الكلي،محتوى التانين والسعرات الحراريه. أظهرت النتائج أن متوسط محتوى الرطوبة و الرماد 11.01 %و 2.06%، قيمة الأس الهيدروجيني 5.06، الدوران الضوئي النوعي +96.4، اللزوجة الضمنية 11.06 سم^{3}جم$^{-1}$، محتوى النيتروجين 0.06%، محتوى البروتين 0.4%، الوزن المكافئ الحمضي 1869، حمض اليورونيك الكلي 10.4% و محتوى التانين 0.04% والسعرات الحراريه 4.064ك كالورى/جرام.

التحلل الحمضي لعينات صمغ السنط (أكيشيا نايلوتيكا صنف أبوعريضه) متبوعاً بقياسات كروماتوغرافيا السائل ذات الأداء العالي كشفت أن محتوى السكر كان كالآتي: أرابينوز 52%، جالاكتوز 24% وكميات قليله من الرامنوز .و أظهرت دراسة الأيونات الموجبة للفلزات باستخدام تقنية بلازما الحث المزدوج أن الكالسيوم لديه أعلى قيمة بين الأيونات الموجبة التي دُرست ($3x10^{-1}$ %) يليه الماغنيسيوم ($5x10^{-2}$ %) ، البوتاسيوم($4.5x10^{-2}$ %) ، الصوديوم ($4x10^{-2}$%) ، الحديد ($5.8x10^{-3}$%) ، الأسترانشيوم ($4 x10^{-3}$%)، المنجنيز ($3x10^{-3}$%)، القصدير

($2.4x10^{-3}$%)، الزنك ($2x10^{-3}$%)، النحاس ($5.4x10^{-4}$%)والنيكل ($9x10^{-5}$%) على الترتيب.

تم قياس توزيع الوزن الجزيئي عن طريق تجزئة الصمغ باستخدام تقنية تشتت ضوء الليزر متعدد الزوايا . أظهرت النتائج ثلاث مكونات رئيسية هى الأرابينوجلاكتان بروتين، الأرابينوجلاكتان و الجلايكوبروتين. تم حساب الوزن الجزيئي من نتائج قياسات التشتت الضوئي باستخدام تقنية نفاذية الهلام المدمج– تشتت ضوء الليزر متعدد الزوايا. وقد وجد أن متوسط الوزن الجزيئ2.54×10^6 دالتون ومتوسط نصف قطر التدوير 36.6 نانوميتر.

أظهرت دراسات الاستحلاب لصمغ السنط (أكيشيا نايلوتيكا صنف أبوعريضه) ثباتية استحلابية ممتازة مماثله لصمغ النايلوتيكا صنف نايلوتيكا وصنف تمنتوزا.

أظهرت الدراسة الريولوجية لصمغ السنط (أكيشيا نايلوتيكا صنف أبوعريضه) أن سلوك الأنسياب له هو قص استرقاقي تحت تأثير معدل القص المنخفض ويسلك سلوك الموائع النيوتونية تحت تأثير معدل القص المرتفع و التراكيز المرتفعة. كشفت نتائج الاختبار التذبذبي لمحاليل الصمغ ان سلوكه يطابق سلوك السوائل النيوتونية.

LIST OF ABBREVIATIONS

GPC-MALLS	Gel Permeation Chromatography Multi angle laser light scattering
JECFA	The joint Expert Committee of Food additives of the FAO/WHO
FAO	Food and Agriculture Organization of the United Nation
WHO	World Health Organization
d[0.1]	The particle diameter, which covers 10% of the particles
d[0.5]	The particle diameter, which covers 50% of the particles
d[0.9]	The particle diameter, which covers 90% of the particles
O.D.O	Octanoic/Decanoic acid triglyceride oil
VMD	Volume Median Diameter
ATST	Accelerated Temperature Stress Test
GPC	Gel Permeation Chromatography
ESI	Emulsion Stability Index
AGP	Arabino Galactan Protein
AG	Arabinoglactan
GP	Glycoprotein
HIC	Hydrophobic Interaction Chromatography
SEC	Size Exclusion Chromatography
IEC	Ion Exchange Chromatography
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry

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CHAPTER ONE

1. Literature Review

1.1 Introduction

In general gums are soluble in water and have different characterization such as colloidal, high molecular weight polymeric compounds and sticky. Moreover, gums can be dried as noncrystallized with fragile mass. (Glicksman, 1973; Laaman, 2011). It is a natural hydrocolloid and is extensively used as an emulsifier/stabilizer in beverage emulsions (Tan, 2004; Mc Clements, 2005). Gums are defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as: "a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (Fam. *Leguminosae*)" (FAO 1999).

Gums is a complex mixture of polysaccharides, protein and arabinoglacto protein species. It has been shown to be highly heterogeneous and is found in nature as mixed calcium, magnesium, potassium and sodium salts of a polysaccharic acid (arabic acid) and other heavy elements (Anderson, *et al*, 1983, Osman, *et al*, 1993, and Islam, *et al*, 1997). The most fundamental property of a gum therefore is its water solubility and high viscosity in aqueous dispersions. For this reason, resins, latexes and other hydrophobic gums are not regarded as true gums. Among the advantages of natural gums over their synthetic counterparts are their biocompatibility, low cost, low toxicity (ecofriendliness) and relative widespread availability (Odeku, 2005; Emeje *et al.*, 2009; Nep and Conway, 2010; Ogaji and Okafor, 2011).

A.nilotica species is widely distributed in subtropical and tropical Africa, from Egypt and Mauritania to South Africa, and also found in Burma, Sri Lanka, Saudi Arabia, West and East Sudan and in the Indian subcontinent Brenan (1983). *A. nilotica* produces a clear gum highly soluble in water. Very few analytical data about the relevant structural features of the polysaccharide in *Acacia nilotica* gum have been reported (Anderson *et al*., 1966, Chal *et al*., 1968, Anderson *et al*., 1977, Karamalla, 1999, and Al-Assaf *et al*., 2005).

1.2 Plant Gums

Plant gum exudates are defined as water soluble or insolubie, complex acidic polysaccharides that are extracted from marine and land plants (either spontaneously or after mechanical injury), that possess the ability to contribute viscosity or gelling ability to dispersions (Abu Baker, Tahir and El-Kheir, 2007). Various plant species yield the complex polysaccharides commercially known as 'plant based gums'. Studies on several of these (mainly plant gum exudates and seed gums) has given rise to the identification of valuable natural sources of complex carbohydrate polymers with attributes which make them of use for industrial applications (Mirhossein and Amid, 2013).

The term gum often describes materials which affect sense of touch, taste and sight in measure summed up as property of gummosis" which is difficult to define but visual and manual examination of the material may cause the observer, to call it gum (Mantell, 1947). Gum harvested from *A. senegal* is referred to as Hashab, while that harvested from *A. seyal* is known as Talha. They are both recognized by the Codex Alimentarus and have been assigned the food additive code E414 (UNCTAD, 2009; Abdel-Gadir, *et al*., 2014).

1.3*Acacia* Genus

Acacia is one of the largest vascular plant genera and encompasses a wide range of ecological environments (Abdel-Farida, Shededa and Mohameda, 2014) species of which can be found in Australia, Africa, India and America (Browna, Warwick and Prychid, 2013). The environmental conditions experienced in these countries range from arid deserts to tropical climates (Browna Warwick and Prychid, 2013). Botanically speaking, the genus is a part of the *Mimosoideae* subfamily, which is a large pantropical sub-family (Abdel-Farida, Shededa and Mohameda, 2014). Its name is derived from the Greek word for thorns '*akakia*' (Borzelleca and Lane, 2008; Chiveu *et al*., 2008).

There are more than a thousand species of *Acacia*, which are classified into three major groups; subgenus *Acacia* (*gummiferae*) containing ~120 - 30 species; subgenus *Aculeiferum* (*Vulgares*) with ~180 - 190 species and subgenus *Phyllodinae* with more than 900 species (Ali *et al*., 2012). According to Coppen, (1995), *Acacia senegal* is generally regarded as having four different varieties namely *A. senegal* (L.) Wild. var.

senegal (syn. *A. verek* Guill.); *A. senegal* (L.) Willd. var. *kerensis* Schweinf; *A. senegal* (L.) Willd. var. *rostata* Brenan; and *A. senegal* (L.) Willd. var. *leiorchis* Brenan (syn. *A. circummarginata* Chiov.); while *Acacia seyal* has two varieties: *A. seyal* Del. var. *seyal* and *A. seyal* (Del.) var. *fistula* (Schweinf) Oliv.

However, there are more than 1100 botanically known species of *Acacia* distributed throughout the tropical and subtropical areas of the world most commercial gum Arabic is derived from *Acacia senegal* locally known as hashab gum (in the Sudan) and as Kordofan, most research works on *Acacia* gums worldwide have been directed towards *Acacia senegal* and to a lesser extent, *Acacia seyal*. Other *Acacia* gums have received very little attention like gum from *Acacia nilotica* and subspecies.

1.3.1 *Acacia* Genus of the Sudan

Sudan is endowed with more than 30 *Acacia* species, most of which yield gum (EL-amin, 1990), these species include, *Acacia senega*l (Hashab), *Acacia seyal* (Talha), *Acacia polyacantha* (Kakamut), *Acacia laeta* (Shubahi), *Acacia mellifere* (Kitir), *Acacia nilotica* (sunt), *Acacia sieberiana* (kuk), and *Acacia nubica* (Lao't) (Abdel Nour, 1999).

1.3.1.1 *Acacia nilotica* (L.) Del

Acacia is derived from Greek word means medical tree, nilotica means found near the Nile River. *Acacia nilotica* is a taxonomic synonym of *Vachellia nilotica*, other common names are: gum Arabic tree, Egyptian thorn, Prickly *Acacia*, and Sunt tree. *Acacia nilotica*, also known as *Mimosa nilotica*, and is known in the Sudan as *Garad*.

Acacia nilotica originated from Africa, the Arabian Peninsula and the Indian subcontinent (USDA, 2012). It is now, commonly, found or cultivated within 30°N and 20°S in almost all tropical and subtropical areas of Africa, Asia, Australia and the Caribbean (Fagg *et al*., 2005; Orwa *et al*., 2009; Ecocrop, 2012).

Babul (*Acacia nilotica* (L.) Willd. ex Delile) is a medium sized, thorny, nearly evergreen tree that can reach a height of 20-25 m but may remain a shrub in poor growing conditions (Fagg *et al*., 2005; Orwa *et al*., 2009;Ecocrop, 2012). The trunk is short, thick (1 m in diameter) and cylindrical, covered with grey bark. The crown may be flattened or rounded. The root system depends on the growing conditions and subspecies: a deep taproot in dry conditions and extensive lateral roots in flooded

conditions. The leaves are 5-15 cm long, alternate and compound with 7 to 36 pairs of elliptical, 1.5-7 mm long x 0.5-2 mm broad, grey-green, hairy leaflets. Flowers are sweetly scented and bright to golden yellow in colour. The fruits are linear, flattened, narrow indehiscent pods, 4-22 cm long and 1-2 cm broad, dark-brown to grey in colour and glabrous or velvety. The pods contain 8 to 15 elliptical, flattened bean-shaped dark seeds (Fagg *et al*., 2005; Cook *et al*., 2005; Orwa *et al*., 2009).

There are two groups of *Acacia nilotica* subspecies. The first group (*nilotica, tomentosa, cupressiformis, indica*) consists of tall riverine trees that grow in seasonally flooded areas. Their pods have a characteristic "necklace" shape with constrictions between the seeds. The second group (*adstringens, kraussiana, leiocarpa, subalata*) grows in drier areas and has straightedged pods (Ndoye-Ndir *et al*., 2008).

A.nilotica is a polymorphic species and is considered to be represented by nine subspecies distinguished by the pubescence of the pods and the shape of the tree; these are: *indica, kraussiana, leiocarpa, nilotica, subalata* and *tomentosa* in Africa, *cupressiformis* and *hemispherica* in India, and *adstringens* in Africa and India (Brenan, 1983).

In Sudan, there are three sub-species, namely, *nilotica, tomentosa* and *adstringens* (Sahni, 1968), *nilotica* is characterized by necklace-like pods narrowly constrict between seeds and glabrous, generally distributed along the White Nile from Jebelein northward and western Sudan (Maydell, 1990). *tomentosa* is distinguished by its necklace-like pods narrowly and regularly constricted between seeds, and is distributed along the Blue Nile south of Sennar, whereas, *adansonii* is distinguished by broad pods slightly constricted between the seeds and is distributed in Darfur and Kordofan (El-Amin, 1990).

1.3.1.1.1 Botanical classification of *Acacia nilotica*

Kingdom: *Plantae*

Class: *Magnoliopsida*

Order: *Fabales*

Family: *Leguminosae*

Subfamily: *Mimosoideae*

Tribe: *Acacieae*

Genus: *Acacia*

Species: *Acacia nilotica* (L.) Willd. ex Delile.

Subspecies: *A. nilotica* subsp. *Adstringens*, *A. nilotica* subsp. *nilotica*, *A. nilotica* subsp. *Tomentosa*, *A. nilotica* subsp. *Subalata.*

Synonymes: *Acacia nilotica* (L.) Willd. ex Delile, *Acacia arabica* (Lam.) Willd. *Acacia scorpioides* W.Wight, *Mimosa arabica* Lam., *Mimosa nilotica* (L.), *Mimosa scorpioides* (L).

Latin name: *Acacia nilotica*

English name: Egyptian thorn, red thorn

Indian name: Babul

Arabic name: Sunt (tree), Garad (fruit). (EL Amin, 1977and Voget, 1995).

1.3.1.1.2 Description of *Acacia nilotica* var. *adstringens*

Acacia nilotica var. *adstringens* is evergreen tree (Fig 1.1); the bipinnate leaves are alternate. The leaflets are ovate and have entire margins. The young branch lets in this sub-species are very hairy or tomentose and only rarely pubescent. The arrangements of the pods differ with other species, they are not necklace like, have no beaded pods, margins distinctly and often irregularly crenate and its width varies from 1.2-2.2 cm. The surface of the pod is densely tomentose. *A. adstringens* produces flowers which are arranged in bright yellow orange globose in florescences, such as this shoot. The shrubs produce legumes (source: Indian Council of Foresty Research and Education).

Fig 1.1: *Acacia nilotica* var. *adstringens* tree, Flowers, leaves and pods

Fig 1.2: Fruits and seeds of *A. nilotica* ssp. *adstringens* from Senegal; photo: © L. Toussaint, www.mauritanie-decouverte.net

1.3.1.1.3 Distribution of *Acacia nilotica* var. *adstringens*

A. nilotica sub-species *adstringens* is widespread in the northern part of tropical Africa from Senegal and the Gambia to the Sudan and extending southwards to Cameroun in the west and Somalia in the east. Specimens of subsp. *adstringens* have been collected in Libya and Algeria but whether they were from indigenous trees or not are uncertain. It is recorded also from Pakistan (Sind) and India (Berar, Bombay) (Ali, 1973). These specimens differ from those from Africa in having glabrescent branch lets. Ali (1973) also records it from Arabia but the material is inadequate for certainty. Ali & Qaiser (1980) reported that subsp. *indica* and subsp. *hemispherica hybridise* and that backcrosses with the parents produce plants similar to subsp. *adstringens* and subsp. *subalata*. This may well be the explanation of these taxa in Asia. *A.nilotica* sub-species *adstringens* is relatively common in west sahelian Africa, as far as Sudan in Darfur and Kordofan, and widespread in northern parts of tropical Africa in Libya and Algeria. It is more adapted to dry conditions, is only found widely dispersed and in dry areas, in the delta of flood zones where population of *tomentosa* have established. Also occurs in wooded grasslands, savannas and dry scrub forests above the flood plains.

1.3.1.1.4 Uses of *A. nilotica*

Acacia nilotica established as very important economic plants since early times as source of tannins, gums, timber, fuel and fodder. They have significant pharmacological and toxicological effects In Africa and the Indian subcontinent; *A. nilotica* is extensively used as a browse, timber and firewood species (Gupta 1970, Mahgoub 1979, New 1980). The bark and seeds are used as a source of tannins (Shetty 1979, New 1980). The species is also used for medicinal purposes. Bark of *A. nilotica* has been used for treating hemorrhages, colds, diarrhea tuberculosis and leprosy while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions (New 1980). The gum of *A. nilotica* is sometimes used as a substitute for gum Arabic (obtained from *A. senegal*) although the quality is inferior (Gupta 1970). Indian Gum is sweeter in taste than that of the other varieties and is used in paints and medicine. The species is suitable for the production of paper and has similar pulping properties to a range of other tropical timbers (Nasroun 1979). The dark brown wood is strong, durable, nearly twice as hard as teak, very shock resistant

and is used for construction, tool handles and carts. It has a high calorific value of 4950 kcal/kg, making excellent fuel wood and quality charcoal. It burns slow with little smoke when dry. It has a 25% more shock resisting ability than teak. *Acacia nilotica* leaf is very digestible and has high levels of protein. Micronutrients, with the exception of sodium, are adequate for animal requirements. Leaves and pods contain 8% digestive protein (12.4% crude protein), 7.2 MJ/ kg energy and are rich in minerals and generally used for feeding sheep and goats in certain parts of India and also very popular with cattle. Pods are best fed dry as a supplement not as a green fodder. The bark contains high levels of tannin (12-20%) that is used for tanning leathers. The tannin also contributes to its medicinal use as a powerful astringent.

The bark of *Acacia nilotica* (booni) tree is useful in the treatment of eczema. In India, The leaves are effective in the treatment of conjunctivitis; *Acacia nilotica* gum allays any irritation of the skin and smoothes the inflamed membranes of the pharynx, alimentary canal and genito urinary organs. In treating tonsillitis, a decoction of the *Acacia nilotica* bark mixed with rock salt can be used as a gargle. In treating leucorrhoea, the decoction of the bark of the *Acacia nilotica* should be used as vaginal douche for the treatment of this disorder. The fresh pods of *A. nilotica* tree are effective in treating sexual disorders. The pods are reported helpful in removing catarrhal matter and phlegm from bronchial tubes.

African Zulu take bark of *Acacia nilotica* for cough treatment (Sonibare *et al*, 2008). The flowers are reported to reduce the body temperature (Anis *et al*, 1994). Masai people/tribe believes *Acacia nilotica* is a good aphrodisiac and the root is said to cure importance. The bark or gum of the plant *Acacia nilotica* is used in West Africa to treat cancers and/or tumours of ear, eye or testicles. It is also used in West Africa to treat indurations of liver and spleen, condylomas and excess flesh. In Senegal, the bark, leaves and young pods are chewed as an antiscorbutic. The bruised leaves are poulticed and used to treat ulcers. In Lebanon, *Acacia nilotica* is infused with orange flower to treat typhoid convalescence. The Chipi and Tonga people / tribes use the root to treat tuberculosis. The Egyptians believe that diabetics may eat unlimited carbohydrates as long as they consume powdered pods of *Acacia nilotica*. The Italian Africa uses the bark concoction in treating small pox. In Ethiopia, *Acacia nilotica* (booni) is used as a lactogogue (increase milk supply). Different parts of this plant such as the leaves, roots, seeds, bark, fruits, flowers, gum and immature pods act as anti-cancer, antimutagenic, spasmogenic, vasoconstrictor, anti-pyretic, anti-

sthamatic, cytotoxic, anti-diabetic, anti-platelet agregatory, antiplasmodial, molluscicidal, anti-fungal, inhibitory activity against Hepatitis C virus (HCV) and *human immunodeficiency virus* (HIV)-I and antioxidant activities, antibacterial, anti-hypertensive and anti-spasmodic activities, and are also engaged for the treatment of different ailments in the indigenous system of medicine (Ali *et al*, 2012).

In Australia, *Acacia nilotica* bark is believed to be an astringent with high tannic acid contents that help to check bleeding, discharge and excess mucus. The extract from this highly astringent herb may block the body's pain triggers. In Ayuvedic medicine, the plant bark or pods are used internally to treat dysentery, chronic diarrhoea and excess mucus .(Ameh *et al*, 2010) Externally, it helps to stop nose bleeding and good for the treatment of hemorrhoids, skin eruptions, leg sores, mouth ulcers, sore throats and dental infections. In ayurveda, *Acacia nilotica* is considered a remedy for premature ejaculation.

Acacia adstringens is used for firewood and good quality charcoal in the Sahelian regions. It has hard heavy heartwood, which resistants water and termites. It used in construction work, boat-building, fencing, tool handles and art objects. It is a source of gum and tannin, the gum is locally used for making ink. The subspecies is, commonly, planted as shade tree. Young bark a source of fiber. The foliage and pods are eaten by camels, sheep and horses. In Burkina Faso, the leaves are used against diarrhea, the grilled and crushed seeds for treatment of hemorrhoids and gingivitis, and the powdered bark as a local aerostatics. A decoction of the pods is used for coughs, and swallowing the juice formed by chewing pods alleviates the rawness of a dry cough. The flowers are a source of pollen and nectar for bees (Wickens *et al*. 1995).

1.3.1.1.5 Phyto-Chemical constituents of *Acacia nilotica* tree

Phytochemical analysis of the aerial parts of the plant demonstrated the presence of flavonoids and polyphenolic compounds in the flowers, tannins, glycosides, volatile oils, organic acids, coumarins, rutin (quercetin 3-Orutinoside) and carbohydrates in the fruits (El-Shanawany, 1996). Malviya *et al* (2011) who reported the presence of saponins, tannins, and flavonoids in the aqueous extracts of *Acacia nilotica* roots, stem, leaves and seeds. Also, Banso (2009) reported that the ethanolic extract of stem bark of *Acaica nilotica* contains saponins and tannins. The concentration of Tannin in

a bark varies considerably from 12-20%. Several polyphenolic compounds have also been reported in a bark (Khare, 2007). Phytochemical screening of the stem bark reveals that it contains terpenoids, alkaloids, saponins and glycosides. Ali *et al.* (2012) reported that *Acacia nilotica* is a medicinal plant acknowledged to be rich in phenolics, consisting of condensed tannin and phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, (+)-catechin, (-) epigallocatechin- 7-gallate and (-) epigallocatechin-5, 7-digallate. Rashid *et al* (2014) reported that the absence of steroids and flavonoids in a bark of *A.nilotica* in their study. But it have a variety of phytochemical such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7- diglucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin- 6,8-bis-Cglucopyranoside, m-catechol and their derivatives. It contains gallic acid, m-digallic acid, (+)-catechin, chlorogenic acid, gallolyated flavan-3, 4-diol, robidandiol (7, 3, 4, 5- tetrahydroxyflavan-3-4-diol), androstene steroid, D-pinitol carbohydrate and catechin-5-galloyl ester. The bark is rich in phenolics viz. condensed tannin and phlobatannin, gallic acid, protocatechuic acid pyrocatechol, (+) - catechin, (-) epigallocatechin-7-gallate, and (-) epigallocatechin-5, 7- digallate. The bark is also reported to contain (-) epicatechin, (+) dicatechin, quercetin, gallic acid, (+) leucocyanidin gallate, sucrose and (+) catechin-5-gallate. T1 (Butanol solubles) and T2 (Butanol insolubles) polymeric tan fractions, both are mostly responsible for the tanning potency of babul liquor and have to some extent good affinity towards hide powder. Due to its lower molecular weight of polyphenols of the bark; mainly; responsible for fungi toxic activity (Anonymous, 2003).

1.4 Gummosis

Gummosis is a common response to wounding, that results in the exudation of a plastic gum sealant from wounds caused as a reaction to external stimuli such as adverse weather conditions, pathogen attack, predation or mechanical damage (Joseleau and Ullmann, 1990).

Ballal *et al*., (2005b) observed that both the synthesis and exudation of gum Arabic occurs only during dry conditions, (i.e. during the dry season in arid climates). However, the physiological and environmental factors controlling gum yield are still not well understood (Abib *et al*., 2012; Harmand *et al*., 2012).

The systematic wounding of a plant to induce gummosis is refered to as “tapping”, and influences gum yield through timing and the tapping intensity. Once tapped, the gum slowly collects in the wound within a 3 - 8 week period, depending upon the weather condition, and a yield of up to 7,000 g can be obtained per tree, on an annual basis (Ballal, *et al.*, 2005a).

1. 4.1 The gum belt

Widespread in tropical Africa from Senegal in the west to Ethiopia and Somalia in the north-east, southwards to Natal; also extends into India. Sudan is known to have a higher density of *A. Senegal* with a uniform distribution of the tree in pure stands spread over fast geographic area places the country as the major producer of gum Arabic in the world (Beshai,1984). In Sudan gum grows in a broad band known as the ‘‘Gum belt’’ lies within the arid and semi-arid zone of mainly 520,000 km^2 in area that extends across Central Sudan between latitudes 10^o and 14^o N, accounting for one fifth of the country's total area (IIED and IES 1990). This belt covers parts of Kordofan, Darfur, Eastern Sudan and Blue Nile and Upper Nile (Hamza, 1990).

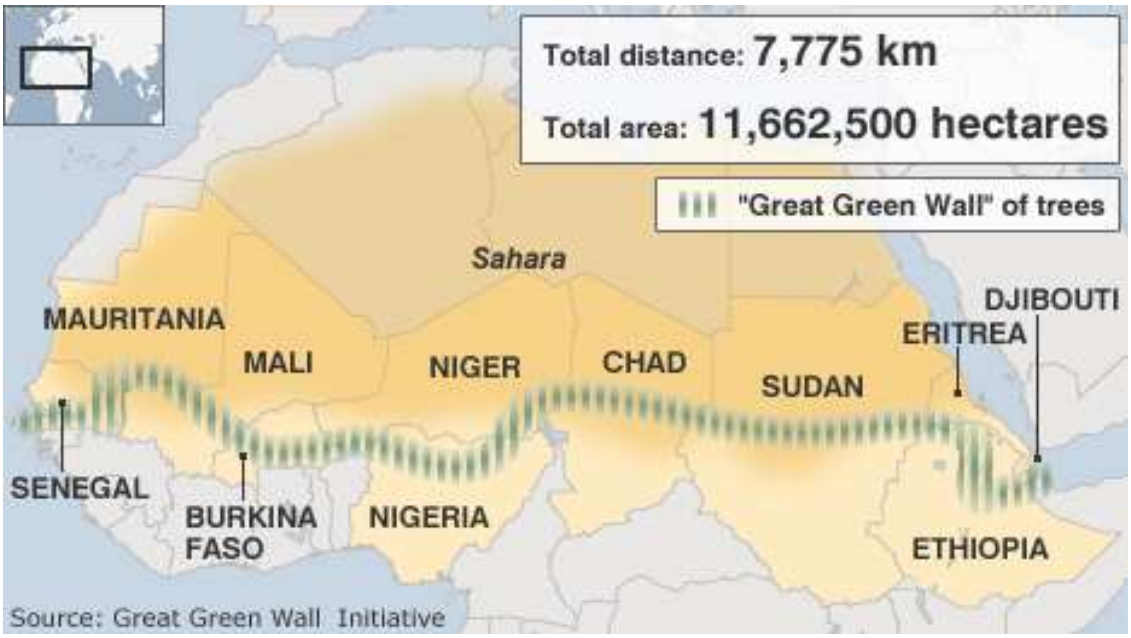


Figure 1.3: ‘Great green wall’ of trees in Africa showing the countries where gum Arabic trees (*Acacia senegal* and *Acacia seyal*) are found both as commercial plantations and in the wild (Adapted from: Green Planet, 2015).

Fig (1.4): gum belt in Sudan

1.4.2 Gum collection in Sudan

Gum Hashsb is collected from *Acacia Senegal* by tapping, whereas all gum talha from *Acacia seyal* is collected as a result of natural exudation. Tapping begins when the trees are just starting to shed their 13 leaves, around the end of October or the beginning of November in Sudan. In order to reach this stage, trees have to grow for a period of 3 to 7 years depending on the method of establishment. Again in the Sudan, there are two tapping seasons, an earlier one before the onset of the colder weather which is between the months of December and March and a later one in the dry spell after the month of March.

After tapping, exudation occurs gradually forming a hard but slightly elastic nodule. As more gum exudes the outer skin expands or cracks and the nodule grows to about 15-30 mm in diameter. When the outer casing becomes so hard that the liquid cannot force it to expand any further, the nodule is ready for picking. The time taken to reach this stage is from 3-6 weeks and as soon as the nodules are picked, new ones start to form and within 10-15 days a second picking is possible. Several branches are

treated in this manner at one tapping. In the following years, other branches or the reverse side of the same treated branches are tapped. An average of four pickings is common, up to seven. The nodules are picked by hand and placed in general in a basket carried by the collector (Anonymous, 1970-2008; MNP, 1980).

1.4.3 Gum Processing

According to Islam *et al*., (1997), the commercial grades of gum exudates obtained from *Acacia* species vary considerably in quality. The best commercial grades of gum are defined as being clean, highly water-soluble and give colourless or pale yellow aqueous solutions. The various processes involved in the production and marketing of gum Arabic are presented in Figure (1.5).

Processing can involve: Cleaning; is a process, which is done before undergoing any further process. Cleaning involves the removal of sand, fines (small fragment of gum) and bark.

Kibbling; is a process which pulverization of gum into small fragments. The fragments of kibbled gum are 0.5 – 2 cm in size.

Spray-dried gum, this involve dissolving of kibbled gum, sieving it, centrifuging to remove insoluble material, pasteurization then spraying the solution into fine droplets (by atomization into a stream of hot air, 70– 80^{o}C, which evaporates water) (William, 1990). Roller dried gum. In this process, the gum is dissolved then coated into rollers by a flow of air. Then the dried gum film is scrapped off the roller. The final product is large flakes of gum.

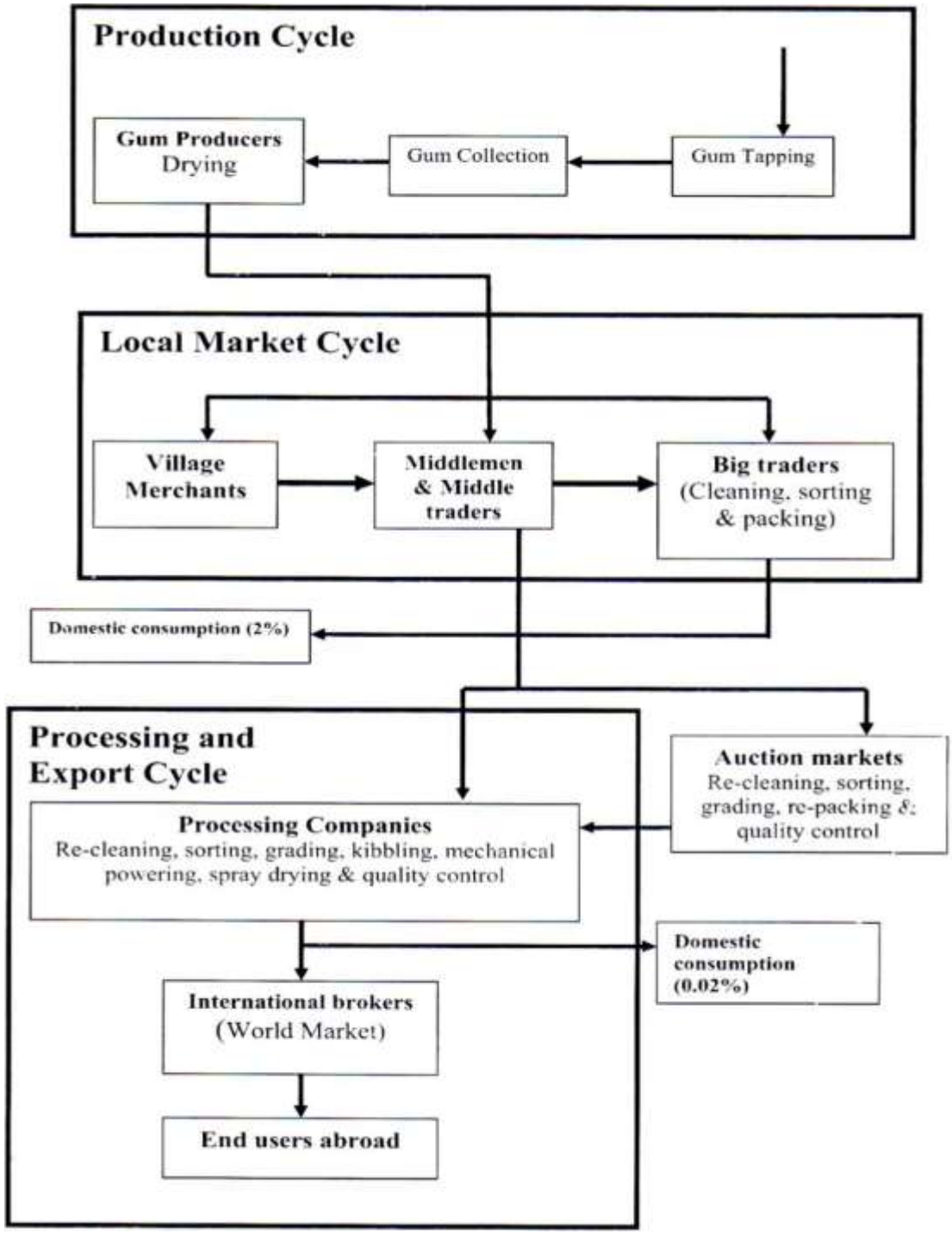
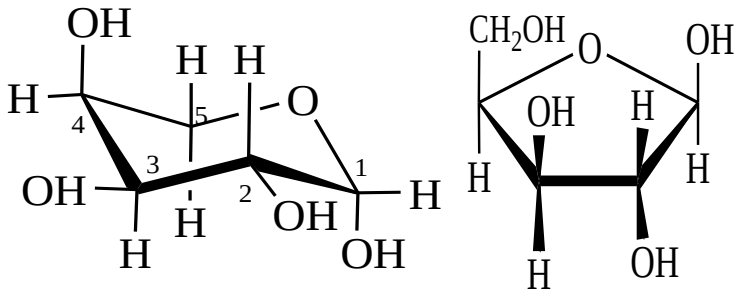


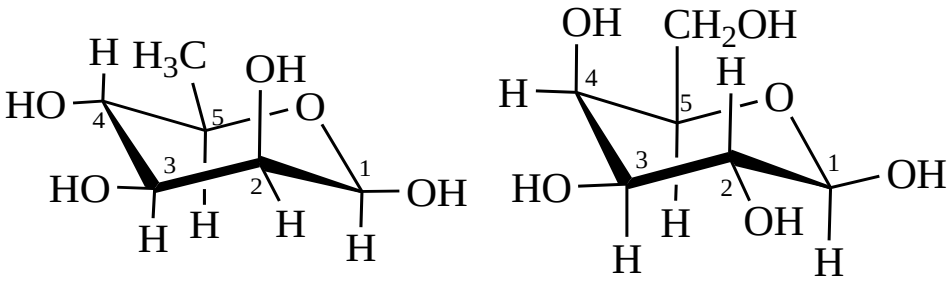
Figure 1.5: Gum production and marketing channel in Sudan (Elrayah, *et al*. 2012). The processes starts with tapping the gum from the tree and allow to solidify (after exposure to air) before collecting it from the plant and subsequently drying it under shade by the gum producers.

1.4.4 Chemical composition of gums

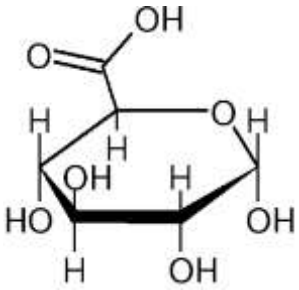
The chemical composition of the gum varies according to the plant source, age of trees, their location and the conditions of the soil (Valnoot *et al*., 2012). Chemically speaking, *Acacia* gums are slightly acidic complex compound, comprised of polysaccharides, glycoproteins and their calcium, magnesium and potassium salts (Flindt *et al*., 2005). It is composed of six carbohydrate moieties; galactopyranose, arabinopyranose, arabinofuranose, rhamnopyranose, glucuropyranosyl uronic acid and 4-o-methylglucuropyronosyl uronic acid (Aspinal *et al*., 1956; Ekhadem, 1956; Glickman, 1979; Sharma, 1981). Polysaccharides that contain arabinose and galactose as their major constituents are called arabinogalactan (AG) (Fincher *et al*., 1983). So all *Acacia* gums chemically are arabinogalactan-proteins (AGP). And it was described as 'heteropolymolecular', i.e., having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer units, in addition to a distribution in molecular weight (Lewis, 1957; Jermyn, 1962; Anderson and Stoddart, 1966). The backbone consists of 1, 3-linked β-D galactopyranosyl units. The side chains are composed of two to five 1, 3-linked β-D-galactopyranosyl units, joined to the main chain by 1, 6-linkages. Both the main and the side chains contain units of α-L-arabino-furanosyl, α-L-rhamnopyranosyl, β-Dglucuronopyranosyl, (Figure 1.6) and 4-O-methyl-β-D-glucuronopyranosyl, the latter two mostly as end-units (Anderson and Stoddart, 1966). The composition is shown in Table 1.1 (Lawson *et al*., 1998).



a- Arabinopyranose Arabinofuranosyl



β- Rhamnopyranose β- Galactopyranose



The α-D form of glucuronic acid

Figure 1.6: Carbohydrates unites in gum molecule.

Table 1.1: Chemical compositions of gum arabic (Lawson *et al*., 1998)

Compound	Composition %
Galactose	36%
Arabinose	30%
Rhamnose	12.6%
Glucuronic acid	19.2%
Protein	2.2%

However, other heavy elements such as Zn, Al, Cd, Cu, Cr, Pb, and Co may also be present but in very small quantities. Gum Arabic is associated with low viscosity and absence of colour, taste and odour (Ibrahim *et al*., 2013). Due to its colourless, odourless, and tasteless properties, it dose not interfere with the blended output. It is insoluble in organic solvents such as alcohol, but water is its main solvent. The high solubility in water makes it versatile in application, since the main solvent is cheap, readily available and non-chemically based (Ademoh and Abdullahi, 2009a). Its pH in a 25% water solution is between 4.1- 4.8 (Creel, 2006). The weak acidity makes it chemically human friendly and non corrosive to tools and equipments. The Gum Arabic has a melting point of 178- 210ºC based on the GA grade (Ademoh and

Abdullahi, 2009). The value is suitable for particleboard manufacture since it can tolerate the high temperature and pressure applied during the particleboard preparation.

Anderson *et al*, (1983) determined the amino acid composition of commercial gum Arabic samples using chromatographic analysis. Moreover, they tried to elucidate the location of amino acids within the molecule. They found that minor amounts of methionine, arginine, isoleucine, lysine, and tyrosine and a considerable portion of the gum's alanine, valine, phenylalanine, and aspartic glutamic acids content exist in the periphery of the Arabinogalactan (AG) blocks.

1.4.5 Molecular structure of *Acacia* gums

Anderson *et al*, (1966b) subjected gum Arabic to smith degradation, using methylation analysis and confirmed that the first degradation to removed uronic acid and rhamnose. The second one removal all of the uronic acid leaving galactose (89%) and arabinose (11%) and this explained that these sugars are found at the periphery of molecule. Then they proposed a structural fragment for the whole gum Figure (1.7).
Street *et al*., (1983) proposed a structure, which is branches composed of 116 galactose units linked by β (1,3) with six branches attached to the chain by (1,6) links The average molecular mass of the structure is about 19.000. Then eventually a model for the whole molecule is obtained, the structure is illustrated in Figure (1.8). Churms *et al.* (1983) also carried out Smith degradation studies; different values for the molecular size and composition of each degradation product were reported.

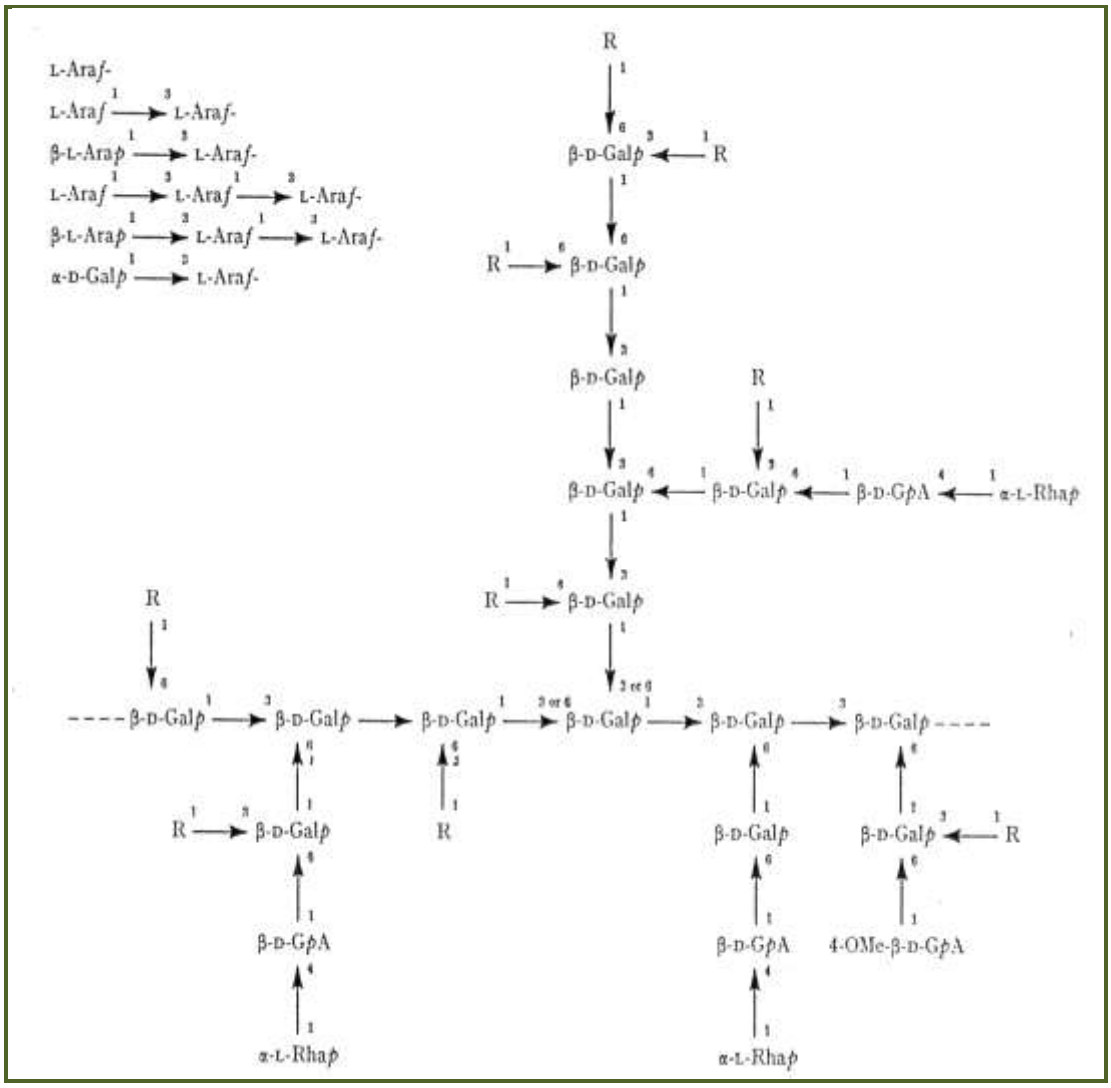


Fig 1.7: Structure Fragment for *Acacia Senegal* gums

(Anderson *et al*, 1966b).

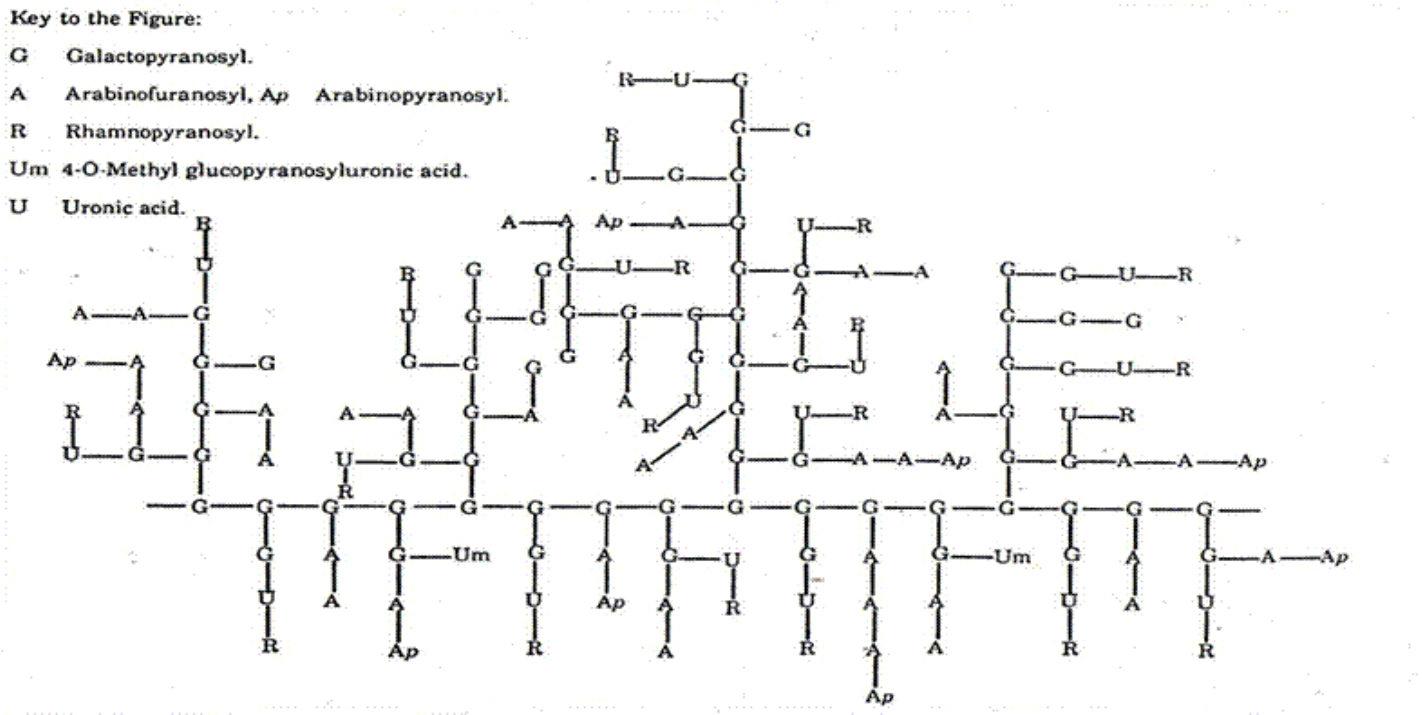


Figure 1.8: Structure of polysaccharide of *A.senegal* (Street and Anderson, 1983)

1.4.5.1 Structural models based on arabinogalactan-protein

The "Wattle-Blossom" model

The essential features of the model are that arabinogalactan blocks of molecular weight 200.000 are linked to a polypeptide backbone thus forming arabinogalactan protein (AGP). Fincher *et al*, (1983) proposed that in any AGP there might be 25 Hyp residues each of which may bear an AG substituent. This AGP substituent is composed of (1>3) linked galactan backbone interrupted by periodate susceptible residues possibly due to Ara or 6-substituted Gal, Fig (1.9).

1.4.5.2 The "Extension like" model

Q *et al*, (1991) proposed a new model for gum Arabic glycoprotein, based on O-galatosyl hydroxyproline at the polysaccharide attachment site. This glycoprotein is equivalent to the AGP described by Connolly *et al*, (1988) but the glycoprotein is basic and deficient in alanine while AGPs are acidic and rich in alanine, this model favors a rod-like molecule in which numerous small polysaccharide substituent's are

regular arranged along a polypeptide backbone, which have an average of 30 sugar residues Fig (1.10). They described the model as a “Twisted hairy rope”.

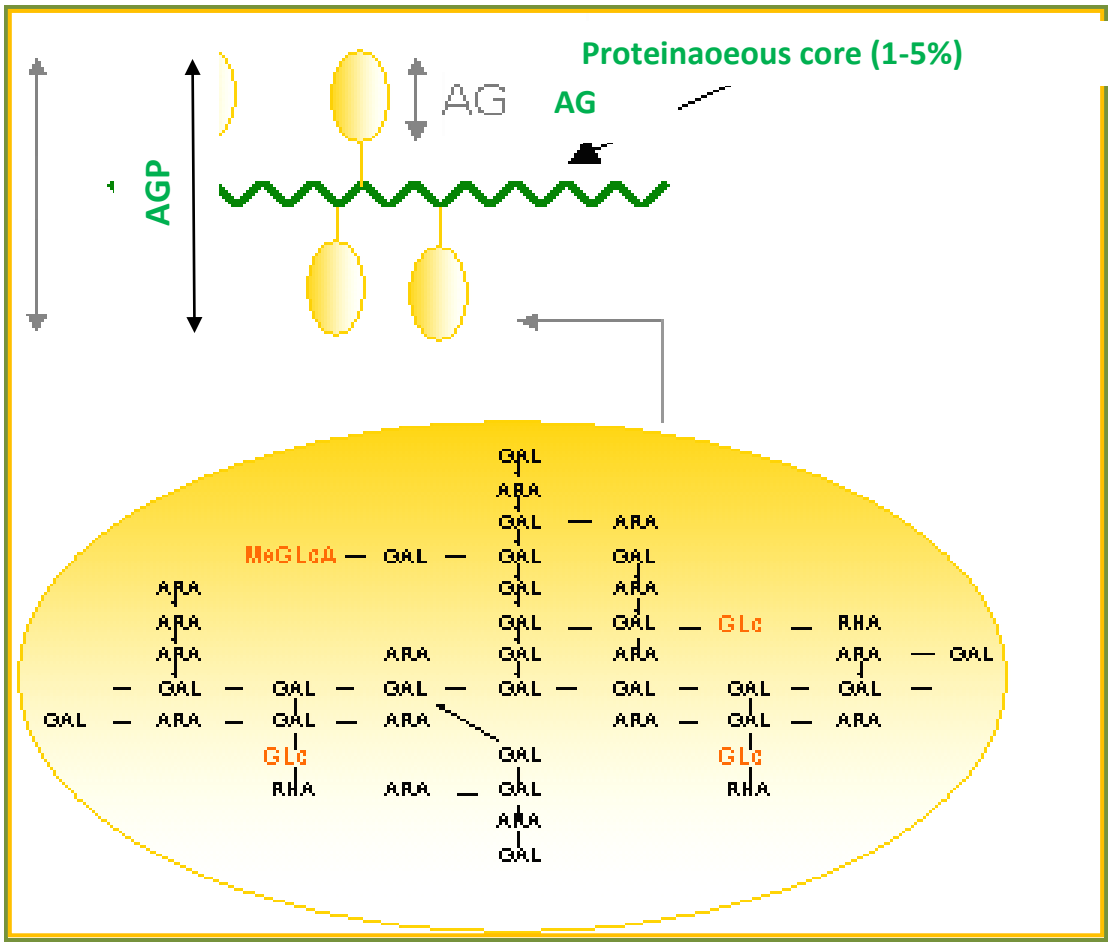


Fig 1.9: Schematic representation of Wattle-blossom model (Fincher *et al*, 1983).

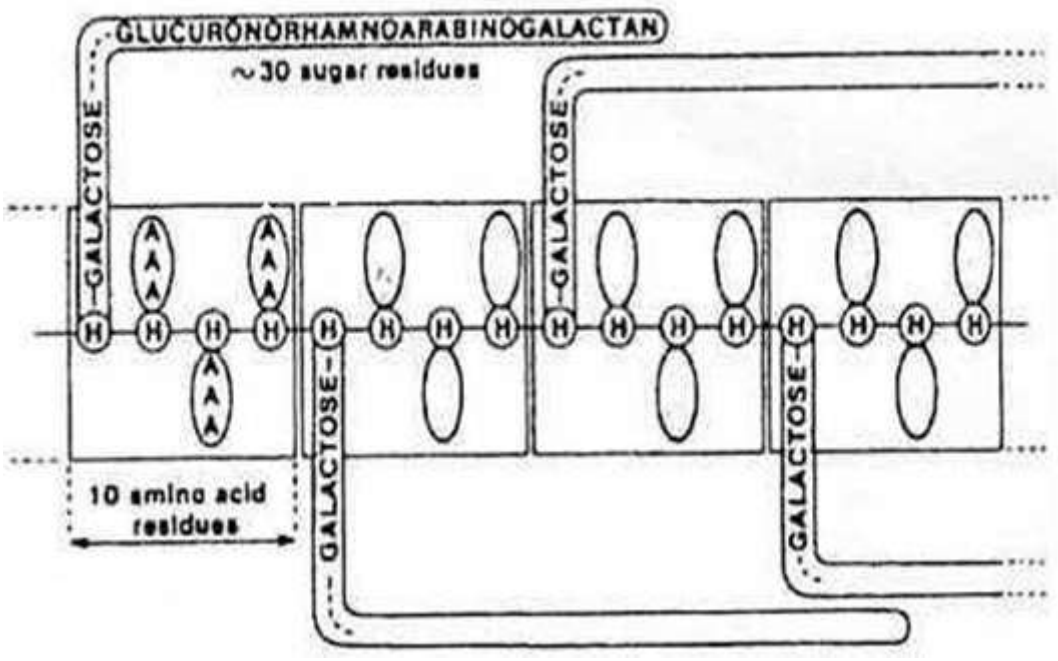


Figure 1.10: "Twisted Hairy Rope" statistical model for the high molecular mass fractions of *Acacia Senegal* gums (Qi *et al*, 1991)

1.4.6 Properties and applications of *Acacia* gums

1.4.6.1 Properties

The most fundamental property of a gum which makes it unique amongst polysaccharide generally is its solubility and viscosity .The majority of gums dissolve in water at different concentration (e. g gum Arabic can form solutions of up to 60% forming viscous solutions). These properties of gums are exploited in many applications.

Acacia gums have excellent emulsifying properties, particularly their AGP fraction. The hydrophobic polypeptide backbone strongly adsorbs at the oil–water interface, while the attached carbohydrate units stabilize the emulsion by sterric and electrostatic repulsion. The heterogeneous nature of the gum makes it an excellent emulsifier. Buffo *et al* (2001) found that stability of beverage emulsions is influenced by a number of processing factors, such as pasteurization and demineralization, and by the pH of the emulsion.

1.4.6.2 Food applications

The use of *Acacia* gums in foods has to be in accordance with the Food and Drug Administration (FDA). *Acacia* gums are mainly used in the confectionery industry, where it is incorporated in a wide range of products. It has a long tradition of use in wine gums, where it produces a clarity that is higher than can be obtained with other hydrocolloids (Williams and Phillips, 2000). Furthermore, it prevents sucrose crystallization, provides a controlled flavor release, and slows down melting in the mouth, making the wine gum long-lasting. It also provides the appropriate texture to these candies, which are easily deformed in the mouth but do not adhere to the teeth. It is also used in chewing gum as a coating agent and as a pigment stabilizer. However, properties such as high solubility associated with low viscosity and absence in taste and odour made it easy to incorporate gum Arabic in different food stuffs without disturbing their organo-leptic properties (Sharma, 1981; Annison *et al*, 1995; Kravtchenko, 1998).

Gum Arabic is extensively utilized in the food industry where it is used as food additive to impart desirable properties through its influence on the viscosity, body and texture of food. In addition, it is nontoxic, odourless, colourless, tasteless and completely soluble in water and does not affect the flavour, odour, colour of the food to which it is added (Tewari, 2010). The absence of toxicity gives it unique characteristics among other food hydrocolloids.

Gum is used in dairy products such as ice creams, packed milk and processed baby foods. It is also used in the backing industry for its comparatively low water absorption

properties and its favorable adhesive properties in glazes and toppings. One of the main uses of spray dried gum Arabic is in solution from such as orange juice, lemon juice, cherry and cola (Buffo, 1952; Phillips *et al*, 1981; Prakash *et al*, 1990). *Acacia* gums are an effective encapsulation agent because of its high water solubility, low viscosity, and emulsification properties and is used in soups and dessert mixes. *Acacia* gums are also used to prevent gelation in canned gravy based pet foods, as they inhibit the extraction of proteins from the meat into the gravy (Glicksman and Farkas, 1975). In food that contains fat and/or oil, gum Arabic is used as emulsifier to maintain uniform distribution of the fat through the product. While as described previously, in the manufacture of soft drinks, it is suitable for use in flavour oil emulsions, where it prevents flocculation and coalescence and inhibits destabilization caused by creaming (Tadesse, Desalegn and Alia, 2007; Wyasu and Okereke, 2012).

1.4.6.3 Non Food applications

Acacia gums used as a protective in pharmaceutical industries due to their unique physico-chemical and functional properties. They are used as a protective colloid in emulsion, as a viscosity improve, as a binding agent and as a coating agent in micro-encapsulation. Microencapsulation is the process whereby particles of an active ingredient are formed and covered with a thin layer of another material, thus providing protection and controlled release. This has been one of the most important uses of gum Arabic in the food and pharmaceutical industries. Gum Arabic is an ideal material in flavour encapsulation due to its emulsifying and surface-active properties. It is used as a fixative in spray drying applications to protect the flavour compound against oxidation and volatilization (Krishnan, Bhosale and Singha, 2005). They are also used as a suspending agent for Colamine suspension; kaolin suspension and cod liver oil emulsion. In medicine gum Arabic has been recommended as an oral laxative (Knight, 1929) and for the treatment of low blood pressure caused by hemorrhage or surgical shock and as intravenous injections for the treatment of nephritic oedema (Osman, 1993). In cosmetics *Acacia* gum are used in lotions and creams to stabilize emulsion, to import viscosity and spreading properties, to add smooth feel to the skin and to form a protective coat. One of the major advantages of *Acacia* gums is that they are not toxic and have no allergic or dermatological reactions (Feinberg *et al*, 1940; Hassan, 2000).

Acacia gums used in pharmaceutical processes should be evaluated regularly for their molecular weight characteristic because in tabletting their binding power increases with molecular weight (Michel *et al*, 1980).

1.4.7 Physico-chemical properties of *Acacia nilotica* gum

Babul gum occurs in the form of rounded or ovoid tears about 1cm in size and the colour varies from pale-yellow to brown or almost black according to the age of the tree and the conditions of collection.

The gum is tasteless and almost completely soluble in water (50g/l00ml). The darker samples contain tannin and are much less soluble, and leave behind a gelatinous residue. *A. nilotica* gum is very slightly dextrorotatory. It contains 13% of moisture and on ignition; it leaves behind 1.8% of ash (CaO, 52.2% and MgO, 19.7%). The gum of *A. nilotica* has a higher molecular weight (Mw, 2.3 x 10^6) than *A. Senegal* (Mw, 600,000) (Sao, 2012). *A. nilotica* gum gave a high, positive specific rotation (+106^o), methoxyl content of (1.05%), and contained only traces of rhamnose (Anderson *et al*., 1966). *A. nilotica* gum gave solutions of low viscosity, and it's unusually low nitrogen content (0.08 %) (Anderson *et al*., 1963, 1966).

Sao (2012), investigations on the structural features of *Acacia nilotica* gum have shown it to contain galactose, L-arabinose, L-rhamnose, and four aldobiouronic acids, viz. 6-O-(β-D-glucopyranosyluronicacid)-D-galactose;6-O-(4-O-mehtyl-β-D-glucopyranosyluronicacid)-D-galactose; 4-O-(α-D-glucopyranosyluronicacid)-D-galactose, and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. The 3, 5, di-O-methyl-L-arabinose and a new crystalline arabinobiose, 2-O-β-L-rabinopyranosyl-L-arabinose ($C_{10}H_{18}O_9$ 1/2 H_2O, mp l03^{o}C) have been isolated from the gum. It also contains arabinobiose, 3-O-β-L-arabLopyranosyl-L-rabinose. The structural evidence suggests that gum molecules possess highly branched galactan frameworks to which are attached uronic acid residues and arabinose-containing side chains.

Seddiqui *et al*, (2015), reported that *A. nilotica* gum exudates collected from ten Indian states, were found to be water soluble at room temperature to form viscous solution, and have pH ranged from 4.21 -4.70, specific rotation from +44.57^o - +80.98^o , moisture content ranged from 2.21 – 6.33% ,ash content from 0.68 – 1.66% and tannin ranged from 0.30 – 14.02%.

1.4.8 Physicochemical properties of*Acacia* gums

The physicochemical properties of the natural gum are most important in determining their commercial value and their use. These properties vary with gums different botanical source, and even substantial differences in gum from the same species when collected from plants growing under different climatic conditions or even when collected from the plant at different season of the year (Hirst *et al.*, 1958).

The most fundamental properties of a gum which makes it unique amongst polysaccharide generally are its solubility and viscosity. The majority of gums dissolve in water at different concentration. Gum Arabic is truly soluble in cold water and form solution of up to 60% forming viscous solution. To identify a particular *Acacia* gum from other gum exudates a number of analytical tests have to be performed (Anderson, 1976).

1.4.8.1 Moisture content

The hardness of gum would be determined by moisture content. The moisture content is weight lost due to the evaporation of water (Person, 1970). It shows the hardness of the gum and hence variability of densities, the amount of densities, and the amount of the air entrapped during formation. The moisture content of good quality gum does not exceed 15 and 10% for granular and spray dried material respectively (FAO, 1999). Anderson and Herbich (1963) reported the moisture content in *A. seyal* gum in the range from 11% to 16.1%. Anderson and Weipping (1991) reported the moisture content in *A. seyal* gum to be within the range of 11.7% to 16.3%. Randall *et al*., (1988) found that the moisture content of Kordofan *A. senegal* 15.5%. Osman (1993) reported the moisture content of *A. senegal* gum was range between 12% to 15%, he reported that the moisture content of *A. senegal* in the average of 13.0%.(Karamallah, 2000), reported the moisture content in different samples of *Acacia* species, had been determined for *A. senegal, A. seyal* and *A. polyacantha* were found to be 7.4%, 7.2 to 8.0% and 6.5% respectively. Study carried out by Karamallah *et al*. (1998) on *A. senegal* samples in season 1994/1995, minimum value of moisture content was found to be 8.1% and the maximum value was 14.05%. In season 1995/1996 minimum value of moisture content was to be 9.15% and the maximum value was 14.3%. Siddig (2003) reported that the moisture content in *A. senegal* was found to be ranged from 10.0% to 16.15%, *A. polyacantha* from 9.4% to11.6% and *A. seyal* 10.4%. Moisture content in *A. seyal* was determined by Hassan (2000), the average value was found to be 8.5% w/w. Omer (2004) reported the moisture in *A. polyacantha* ranged from 5.8% to 10.6%. Moisture content

in *A.senegal* var. *senegal* and *A.seyal* var. *seyal* gum was determined by Abdelrahman (2008) and it was found to be 11.01% and 11.07% respectively. Younes (2009) reported the mean value of moisture content for *A.senegal* var. *senegal* gum as 11.01% and the range was 9.91% to 14.72%, and the mean value of moisture content for *A.seyal* var. *seyal* gum was 10.10% and the range was 9.90% to 10.35%. Ayman (2009) reported that the mean value of *A.senegal*, *A. seyal* and *A.polyacantha* gum was found to be 11.01, 10.10 and 10.48% respectively. Satti (2011) reported the mean value of moisture content for *A.nilotica* var. *nilotica* gum in season 2008 and 2009 ranged from 8.04 to 11.89% with average value of 10.33%, and for season 2010 was ranged between 9.85 and 11.69% with average of 10.18%. Moisture content for *A.nilotica* var. *nilotica* gum was determined by Alobied (2015) and found to be ranged from 9.57 to 11.68%. Siefeldawla (2018) reported average value of moisture content for *A. nilotica* var. *tomentosa* gum as 10.6 and 11.95%.

1.4.8.2 pH

The hydrogen ion concentration is very important in chemistry and industry of gums, therefore functional properties of gum are affected by changes in pH e.g. viscosity, emulsifying power. Crude gum Arabic is slightly acidic because of the presence of few free carboxyl groups of its constituent acidic residues, D-glucuronic acid and its 4-O-methy1 derivatives. Karamalla (1965) reported pH values of 4.42 for *Acacia Senegal* gum while he recorded value of 4.74 for *Acacia seyal* var. *fistula* gum. Anderson (1967) reported values of 4.35 to 4.60 for *Acacia seyal* var. *seyal* gum. Karamalla *et al*, (1998) reported range of 4.38 to 4.89 with the mean value of 4.54 for commercial sample, while in the same study they reported values in the range of 4.04 to 4.48 with sum average value of 4.48. The pH value had been determined by Younes (2009); he reported a value of 4.78 for *A.senegal* var. *senegal* gum and 5.16 for *A. seyal* var. *seyal* gum. Ayman (2009) reported pH value of 4.78, 5.16 and 5.72 for *A. senegal*, *A. seyal* and *A. polyacantha* respectively. Satti (2011) reported pH value for *A. nilotica* var. *nilotica* gum ranged from 5.15 to 5.24. pH value for *A.nilotica* var. *nilotica* gum reported by Alobied (2015) was found to be 5.1.Tthe main values of pH for *A.nilotica* var. *tomentosa* gum reported by Siefeldawld (2018) werefound to be 5.25 and 5.13.

1.4.8.3 Intrinsic viscosity

Viscosity is a measure of the resistance that a fluid offers to an applied shearing force. Studies of flow of gum solutions play an important role in identification and characterization

of their molecular structure. Since viscosity involves the size and the shape of the macromolecule, it was considered as one of the most important analytical and commercial parameter (Anderson and Dean, 1969). Viscosity can be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic viscosity. It is also presented as kinematic or dynamic viscosity.

The intrinsic viscosity has great practical value in molecular weight determinations of high polymers. This concept is based on the Mark-Houwink relation suggesting that the intrinsic viscosity of a dilute polymer solution is proportional to the average molecular weight of the solute raised to a power in the range of 0.5 to 0.9. Values of the proportionality constant and the exponent are well known for many polymer-solvent combinations. Solutions viscosities are useful in understanding the behavior of some polymers.

Two theories were proposed to deal with the theoretical aspects of the viscosity of liquids. The first was proposed by Andrade, (1934) and (1940), which was developed from the assumption that the viscous force arise from the transfer of momentum, as which gases, but with the addition of strong contribution by the molecular forces, the second theory was proposed by Eyring *et al*, (1941), which assume that viscous forces are of a molecular nature, so that the theory of the rate process may be applied.

The coefficient of viscosity of a liquid is measured by a number of methods which include the determination of the rate of flow through a capillary tube, the rate of settling of a sphere in a liquid and the force required to turn one of the two concentric cylinders at certain angular velocity. The most commonly used method to determine the viscosity of a liquid is to measure the time taken by the liquid to flow between fixed marks in a capillary tube under the draining effect of gravity. Viscosity coefficient (η) of a liquid or a solution is then calculated from Poisuille equation;

$$\eta = \frac{\pi.\Delta P.R^4}{8.L.V} t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.1)$$

Where;

Δ P is the pressure difference across the capillary ends.

t is the time of efflux determined by the experiment.

v is the volume of sphere containing the liquid.

R and L are the radius and length of the capillary tube respectively.

The intrinsic viscosity of a solution can be determined from experimental values of Newtonian viscosity in dilute solution using the following standard relationships:-

The relative viscosity (η_{rel}) is the viscosity of the solution (η) relative to that of the pure solvent (η_0) provided that measurements are made with same instrument, that is;

$$\eta_{re} = \frac{\eta}{\eta_0} = \frac{t\rho}{t_0\rho_0} \quad \text{..... (1.2)}$$

Where (t) and (t_0) are the time of efflux of the solution and the pure solvent, and (ρ), (ρ_0) are their densities respectively. The term specific viscosity (η_{sp}) is often used. It is related to the relative viscosity by the relation;

$$\eta_{sp} = \left[\frac{\eta}{\eta_0}\right] - 1 = \eta_{rel} - 1 \quad \text{..... (1.3)}$$

The limit of specific viscosity over the concentration (c) as the concentration approaches zero is called intrinsic viscosity [η] that is;

$$[\eta] = \lim_{c \to 0}\left[\frac{\eta_{sp}}{c}\right] \quad \text{..... (1.4)}$$

Specific viscosity expresses the incremental viscosity due to the presence of the polymer in the solution. Normalizing η_{sp} to concentration gives $\frac{\eta_{sp}}{c}$which expresses the capacity of a polymer to cause the solution viscosity to increase, i.e., the incremental viscosity per unit concentration of polymer. As with other polymer solution properties, the solutions used for viscosity measurements will be non-ideal and therefore $\frac{\eta_{sp}}{c}$will depend on C.

The extrapolated value of$\frac{\eta_{sp}}{c}$ at zero concentration is known as the intrinsic viscosity (η).

Intrinsic viscosity (η) is shown to be a unique function of molecular weight (for a given polymer-solvent pair) and measurements of (η) can be used to measure molecular weight. The remaining form for viscosity is the inherent viscosity. Like η_{sp}, ln η_{red} is zero for pure solvent and increases with increasing concentration, thus ln η_{red} also expresses the incremental viscosity due to the presence of the polymer in the solution.

Normalizing ln η_{red} to concentration or ln $\frac{\eta_{red}}{c}$gives the inherent viscosity in the limit of zero concentration, η_{red} extrapolates the same as $\frac{\eta_{sp}}{c}$ and becomes equal to the intrinsic viscosity.

The intrinsic viscosity (η) is a measure of the hydrodynamic volume occupied by a macromolecule, which is closely related to the size and conformation of the macromolecular chains in a particular solvent (Higiro *et al*., 2007).

Experimental results with polymer solutions has revealed that the slope of the $\frac{\eta_{sp}}{c}$ vs.C curve, k, depends on molecular weight of the polymer. Huggins found that a plot of k versus $(\eta)^2$ was linear and passed through the origin.

The measurement of polymer viscosity dilute solutions are usually used and the intrinsic viscosity can be calculated, from Mark-Houwink equation, by comparing the efflux time (t) required for a specified volume of polymer solution to flow through the capillary tube with the corresponding efflux time (t_0) for the solvent.

Anderson (1966) reported an intrinsic viscosity value of 20cm^3g^{-1} and he found that the oldest and youngest trees gave gums of low intrinsic viscosity. In (1983) he also reported 13.4cm^3g^{-1} for authenticated gum and 17$cm^3g^{-}1$ for commercial samples of *Acacia senegal* gum. Jurasek (1993) reported 13.4 to 23cm^3g^{-1} Karamalla *et al* (1998) studied over 1500 authentic and commercial *A. senegal* they reported that the mean of the intrinsic viscosity was to be 16.44 cm^3 g^{-1}. Karamalla (2000) collected data between 1960 and 1999 for *A. senegal* and *A. seyal* gum in Sudan; he reported intrinsic viscosity 16.6 cm^3 g^{-1} for *A. senegal* and 11.0 cm^3 g^{-1} for *A. seyal*. Hassan (2000) reported intrinsic viscosity value of 14.2$cm^3.g^{-1}$ for *Acacia Seyal* gum. Al-Assaf *et al*., (2005) reported that the intrinsic viscosity of sixty seven samples of *A. senegal* var. *senegal* in the range 9.7 to 26.5cm^3g^{-1}. The intrinsic viscosity of *A. seyal* var. *seyal* gum had been determined by Siddig *et al*., (2005), it was found to be 14cm^3g^{-1}. Omer (2006) found that an average values of intrinsic viscosity equal to 14.6cm^3g^{-1}, and 11.4cm^3g^{-1} for *A. senegal* var. *senegal* and *A.seyal* var. *seyal* respectively. Ibrahim (2006) reported intrinsic viscosity for *A. senegal* was found to be 14.61 cm^3 g^{-1}, while for *A. polyacantha* gum the average was found to be 9.15 $cm^3g^{-1}$and for *A. seyal* it was found to be 11.40 cm^3 g^{-1}. Abdelrahman (2008) reported the average value of intrinsic viscosity of *A.senegal* var. *senegal* gum 15.4cm^3g^{-1} whereas equal to 11.6cm^3g^{-1} for *A.seyal* var. *seyal* gum. The intrinsic viscosity had been determined by Elmanan *et al*., (2008), they reported that the intrinsic viscosity ranged between 14.7 to 17.3cm^3g^{-1} for *A.senegal* var. *senegal* and between 14.6 to 14.9cm^3g^{-1} for *A.seyal* var. *seyal*. Younes (2009) reported a value of 18.9cm^3g^{-1}intrinsic viscosity for *A.senegal* var.*senegal* and 15.5cm^3g^{-1} for *A.seyal* var. *seyal* gum. Ayman (2009) reported that the intrinsic viscosity for *A.senegal* was 18.87cm^3g^{-1}, while for *A.seyal* was 15.47cm^3g^{-1} and for *A.polyacantha* was 18.98cm^3g^{-1}. Satti (2011) reported intrinsic viscosity ranged between 10.19 to 10.65cm^3g^{-1} for *A.nilotica* var. *nilotica*. The intrinsic viscosity of *A.nilotica* var. *nilotica* gum determined by Alobied (2015) was found to be ranged from 8.56 - 11.13cm^3g^{-1}.Siefeldawla (2018) reported average value of intrinsic viscosity for *A. nilotica* var. *tomentosa* gum equal to 10.16 and 11.47 cm^3g^{-1}.

1.4.8.4 Specific Optical Rotation

The optical activity of organic molecules (saccharides and carbohydrates) is related to their structure and a characteristic property of the substance (Stevens *et al.*, 1987). Anderson and Stoddart (1966) reported that the specific rotation for electrodialysed *Acacia senegal* gum was -31, 5º.Pure gum from *A. senegal* has specific optical rotation of -27º to -30º (Tioback, 1922). Certain variation in the degree of the optical rotation (-27º to -32º) has been noticed by (Anderson *et al*, 1968). In 1998 Karamalla *et al.* found that the mean of the specific optical rotation of commercial *A. senegal* gum was -30.54º. Anderson *et al*., (1963) reported the specific optical rotation of *A. seyal* gum in the range from +44^{o} to +56^{o}. Osman (1993) reported specific optical rotation of *A. senegal* to be ranging between -29^{o} to -31^{o}. Omer (2006) reported that an average values of specific optical rotation equal to -32^{o}, and +49.4^{o} for *A. senegal* and *A. seyal* respectively. Abdelrahman (2008) reported the average value of optical rotation of *A.senegal* var. *senegal* gum -31.5^{o} whereas equal to +61^{o} for *A.seyal* var. *seyal* gum. Younes (2009) reported a value of -30^{o} specific rotation for *A.senegal* var. *senegal* and +52^{o} for *A.seyal* var. *seyal* gum. Satti (2011) reported the mean value of specific optical rotation for *A.nilotica* var. *nilotica* ranged from +72.5 to +107.5^{o}. Alobied (2015) found that the mean value of specific optical rotation of *A.nilotica* var. *nilotica* ranged between +88.6 and +98.4 ^{o}.Siefeldawla (2018) reported that an average values of specific optical rotation equal to +97.67^{o}, and +91.79^{o}for *A.nilotica* var. *tomentosa* gum.

1.4.8.5 Ash content

Ash content is a measure of inorganic residue remaining after organic matter has been burnt. The inorganic residues exist as elements. Siddig (1996) explained that the type of the soil (clay or sand) affected the ash content significantly. Study carried out by Karamallah *et al*. (1998) on *A. senegal* samples in season 1994/1995, the ash content ranged from 2.75% to 5.25%, while sample collected in season 1995/1996 for *A.senegal* and *A. seyal* gum in the value of 2.87% and 3.93% respectively. Osman (1993) reported an ash content of *A. senegal* in the average of 3.6%. Anderson *et al.*, (1983) found that the value of ash content of commercial gum arabic to be 4.4%. Later, Anderson *et al* (1991) reported 3.6% Ash content for Sudanese formulations. FAO (1999) reported that the ash content of gum Arabic did not exceed more than 4%. Hassan (2000) reported the averages of the ash content of *A. seyal* to be 0.7%w/w. Siddig (2003) reported that the ash content in *A. senegal* was found to be in the range from 2.0% to 3.0%, *A. polyacantha* from 2.0% to 2.5% and *A. seyal* 3.0%. Omer

(2006) reported the ash content of *A.senegal* var. *senegal* was 3.27% and 2.61% for *A.seyal* var. *seyal* gum. The means value of ash content reported by Abdelrahman (2008) for *A.senegal* var. *senegal* and *A.seyal* var. *seyal* gum in the range of 3.32% and 2.43% respectively. Younes (2009) reported the mean value of ash content for *A.senegal* var. *senegal* gum was 4.89% and for *A.seyal* var. *seyal* gum 4.47%. The mean value of ash content determined by Ayman (2009) was 4.89% for *A.senegal* and 4.47% for *A.seyal* while for *A.polyacantha* was 4.45%. Satti (2011) reported the value of ash content for *A.nilotica* var. *nilotica* gum for season 2008 was ranged between 1.36 to 2.36% with average value of 1.82%, for season 2009 was ranged between 1.4 to 2.35% with average value of 1.84%, while for season 2010 was ranged between 1.79 to 1.99% with average value of 1.91. Alobied (2015) reported that the ash content for *A.nilotica* var. *nilotica* gum ranged between 1.60 and 2.10%. The means value of ash content reported by Siefeldawlw (2018) was 2.17 and 1.94% for *A. nilotica* var. *tomentosa* gum.

1.4.8.6 Nitrogen and Protein content

The role of nitrogen and nitrogenous component in the structure, physicochemical properties and functionality of gum Arabic was recently subjected to intensive investigation (Gammon *et al*., 1968; Anderson *et al*., 1985). In 1988 Dickinson studied the emulsifying behavior of gum Arabic and concluded that there is strong correlation between the proportion of protein in the gum and it is emulsifying stability. Idris (1989) showed that the protein contents of fresh samples were fairly constant (2%), irrespective of the age of the tree. Siddig (1996) reported that the average value of nitrogen content of commercial samples of *Acacia senegal* gum and 80% of authenticated samples analyzed were in the range (0.26 to 0.39%). Karamallah (2000) reported nitrogen content in comparative analytical data for *A. senegal* and *A. seyal* gums collected between 1960–1999 in Sudan to be 0.33% w/w for *A. senegal* gum, and 0.11% w/w for *A. seyal* gum. Osman (1993) reported that nitrogen content for the *A. senegal* gum to be 0.31% and protein content 2.4%. Hassan *et al*., (2005) reported protein content of *A. seyal* had a mean value of 0.96% Omer (2006) determined the nitrogen content for samples of *A. senegal* and *A. seyal* from different locations, the values were 0.35% and 0.14% for *A. senegal* and *A. seyal* respectively, whereas protein content had a value of 2.3% and 0.93 respectively Abdelrahman (2008) reported the average value of nitrogen content of *A.senegal* var. *senegal* gum 0.37% whereas equal to 0.14% for *A.seyal* var. *seyal* gum, he found that protein content of *A.senegal* var. *senegal* gum 2.4% and equal to 0.95% for *A.seyal*

var. *seyal* gum. Recent study by Younes (2009) determined nitrogen content for *A.senegal* var. *senegal* 0.35% and protein content. 2.3%, for *A.seyal* var. *seyal* the author reported nitrogen content 0.22% and protein content 1.4%. Ayman (2009) reported the average value of nitrogen and protein content of *A.senegal* gum is 0.35% and 2.31% and for *A.seyal* is 0.218% and 1.44% while for *A.polyacantha* is 0.38% and 2.5%. The mean of nitrogen content which was reported by Satti (2011) for *A.nilotica* var. *nilotica* gum was 0.02%, while protein content was 0.16%. Alobied (2015) reported nitrogen and protein content for *A.nilotica* var. *nilotica* gum in the range 0.019 to 0.029% and 0.124 to 0.189% respectively. Siefeldawla (2018) reported nitrogen and protein content for *A.nilotica* var. *tomentosa* gum equal to (0.08 to 0.05%) and (0.51 to 0.28%) respectively.

Table 1.2: Analytical data of gum exudates from different *Acacia* species in Sudan. (Karamalla, 1999).

Species	**Moisture %**	**Ash %**	**Nitrogen %**	**Protein %**	**PH**	**Titrable acidty**	**Relative viscosity**	**SP.Rot (degree)**	**Arabinose %**	**Rhamnose %**	**Methoxyl %**
A.Sieberanavar. Sieberana	5.30	1.90	0.35	02.19	3.95	5.82	1.36	+74.16	41	03.0	-
A.Sieberanavar.vermesenii	4.90	2.10	0.35	02.19	3.88	6.00	1.47	+77.16	48	05.0	-
A.nubica	4.60	0.03	0.35	02.19	3.50	10.20	0.50	+64.16	48	04.3	0.15
A.tortilis subsp. Raddiana	4.60	1.80	1.84	11.50	3.60	8.56	0.77	+71.33	43	07.0	-
A.tortilis subsp.spirocarpa	6.40	2.03	1.40	07.50	3.85	6.50	0.76	+68.66	41	08.0	-
A.tortilis subsp.tortilis	6.10	1.90	1.20	08.75	4.15	4.80	0.80	+69.00	58	05.0	0.57
A.drepanndolobium	6.10	0.01	0.87	05.44	4.05	5.10	1.01	+75.83	49	02.0	0.40
A.grrardii	5.90	3.10	2.31	14.44	4.40	3..80	2.75	+48.50	37	09.0	-
A.ehrenbergiana	7.90	2.60	0.22	01.37	3.45	11.00	0.37	+5.66	39	08.0	-
A.nilotica subsp.nilotica	6.10	0.03	0.06	00.37	4.10	5.00	0.69	+97.66	42	01.8	1.14
A.nilotica subsp.tomentosa	5.80	0.04	0.10	00.62	4.48	4.15	0.90	+80.16	4	01.9	0.88
A.nilotica subsp.astringen	5.60	0.06	0.06	00.37	3.75	7.00	0.68	+75.16	39	01.0	-
A.laeta	3.20	2.80	0.51	03.19	3.70	7.70	1.12	-37.50	23	12.0	0.33
A.polycantha	6.50	2.70	0.34	02.12	4.25	4.50	0.66	-19.10	31	09.0	-
A.seyal var.seyal	7.20	2.30	0.10	00.63	4.35	4.18	1.28	+50.50	52	04.0	1.02
A.seyal var.fistula	8.00	1.60	0.14	00.87	3.80	6.90	1.77	+42.66	49	07.0	0.90
A.senegal	7.40	3.70	0.33	01.87	4.66	3.60	1.40	-31.30	21	14.0	0.36

1.4.8.7 Equivalent weight and uronic acid anhydride

Titrable acidity, which is the number of mls of 0.02N sodium hydroxide that neutralize 10 mls of 3% gum solution, represents the acid equivalent weight of gum, from which the uronic acid content can be determined (Karamalla, 1965; Jurasick, 1993). Uronic acids constitute a major component of many natural polysaccharides. They are widely distributed in animal and plant tissues. A number of methods have been developed for determination of uronic acids. They include colorimetric, decarboxylation and acid base titimetric methods. Gums differ widely in their equivalent weight and uronic acid content (Karamalla, 1965). Anderson and Herbich (1963) reported that the uronic anhydride percentage of *A. seyal* sample after electrodialysis was found to be in the range 12.1 to 16.8, while the crude gum in the range 9.0 to 16.4%. Anderson and Weiping (1991) reported the range of 1200 to 1690 equivalent weights for 8 samples of *A. seyal* gum solutions. (1993) reported uronic acid for *A. senegal* was found to be in the range 12 to 28.3% and for *A. seyal* 6.5%. Hence acid equivalent weight found to be in the range 1430 to 1125 and for *A. seyal* 1470. Osman *et al*. (1993a) reported an acid equivalent weight for *A. senegal* ranging from 1040 to 1119. Karamallah *et al*., (1998) reported the mean value of uronic acid for 115 *A. senegal* gum samples collected in season 1994/1995 as 13.7% and a mean value of 1436 acid equivalent weight .Seventy four authenticated *A. seyal* samples have been studied by Hassan *et al*. (2005) from different location by using acid–base titrimetric method, the authors reported that the mean value of acid equivalent weight 1489 and the uronic acid 11.9%. Omer (2006) reported that the acid equivalent weight was to be 1161 in average, and glucouronic acid was to be 15.2% in average for *A. senegal,* whereas acid equivalent weight was to be 1107.9 in average and glucouronic acid was to be 15.9% in average for *A. seyal*. Abdelrahman (2008) reported the value of 16.8% uronic acid of *A.senegal* var. *senegal* gum and 1153.8 acid equivalent weight values. The author found the value of uronic acid of *A.seyal* var. *seyal* 16.4% and 1185.8 acid equivalent weight value. Acid equivalent weight and uronic acid had been determined by Younes (2009), he reported the mean value of acid equivalent weight 1620 and uronic acid 11.89% for *A.senegal* var. *senegal* gum, and also he reported a value of 1180 acid equivalent weight and 16.34% uronic acid for *A.seyal* var. *seyal.* Ayman (2009) reported values of acid equivalent weight and uronic acid for *A.senegal* were 1620 and 11.98% and for *A.seyal* were 1180 and 16.43% while for *A.polyacantha* were 1741 and 11.15%. Satti (2011) reported the mean value of acid equivalent weight for *A.nilotica* var. *nilotica* gum for season 2008, 2009 and 2010 were 1904.48, 1910.61 and 1908.37, while the

mean of uronic acid were 10.2, 10.17 and 10.18% respectively. Alobied (2015) reported the mean value of acid equivalent weight for *A.nilotica* var. *nilotica* gum was 1866.76 while uronic acid was 10.42%. Siefeldawla (2018) reported the mean value of acid equivalent weight for *A.nilotica* var. *tomentosa* gum was 2182.86 and 2187.73 while uronic acid was 8.90 and 8.88%.

1.4.8.8 Sugars composition

Monosaccharide composition of *Acacia* gums is determined by acid hydrolysis of the gum, complete hydrolysis yields four basic sugar constituents; D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid (Sharma, 1981). Osman *et al*. (1993a) studied six commercial sample of *A. senegal* and authenticated sample of *A. seyal*, The authors reported that *A. senegal* gum contain 12-14% rhamnose, 24-29% arabinose and 32-28% galactose. . Jurasek *et al*., (1993) reported sugar composition as 34-46% galactose, 23-35% arabinose and 9-16% rhamnose for *A. senegal*, and 38% galactose, 46% arabinose and 4% rhamnose for *A. seyal*. Karamallah (1999) reported comparative analytical data for *A. senegal* and *A. seyal* gums collected between 1960 and 1999 in Sudan, he reported sugar content had a value of 36-42% galactose, 24-29% arabinose, 12-14% rhamnose and 16-17% glucuronic acid for *A. senegal*, whereas had a value of 37-38% galactose, 41-45% arabinose, 3-4% rhamnose and 11-12% glucuronic acid for *A. seyal*. Islam *et al*., (1997) and Williams *et al*., (2000) reported the sugar content of *A.senegal* var. *senegal* 44% galactose, 27% arabinose, 12% rhamnose and 14.5% glucuronic acid. Also they reported the sugar content of *A.seyal* var. *seyal* as 38% galactose, 46% arabinose, 4% rhamnose and 6.5% glucuronic acid. Siddig *et al*., (2005) reported the sugar content of *A.seyal* var.*seyal* 36% galactose, 44% arabinose, 3% rhamnose and 16% glucuronic acid. The average values of sugar content determined by Abdelrahman (2008) of *A.senegal* var. *senegal* 29.7% galactose, 21% arabinose and 10.1% rhamnose. He also found the sugar content of *A.seyal* var.*seyal* as 28.8% galactose, 34% arabinose and 1.6% rhamnose. Satti (2011) reported the sugar content for *A.nilotica* var. *nilotica* gum as 14.27-19.022% glactose, 31.09 - 49.99% arabinose and 5.48-14.51% rhamnose. Values of sugar content which was determined by Alobied (2015) for *A.nilotica* var. *nilotica* gum 14.73 - 18.63% glactose, 37.67 - 47.18% arabinose and 6.27 - 12.54% rhamnose.

1.4.8.9 Cationic composition

1.4.8.9.1 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Inductively coupled plasma-mass spectrometry (ICP-MS) is an analytical technique used for the detection of trace metals in environmental samples. The primary goal of ICP-MS is to get elements to emit characteristic wavelength specific light which can then measured. ICP-MS is a very high temperature (~ 10000 K) excitation source that efficiently desolvates, vaporizes, excites, and ionizes atoms.

1.4.8.9.2 Principle of ICP-MS

This international standard describes the multi-elemental determination of analytes by ICP-MS in aqueous and nitric acid or aqua regia digests. The method measures ions produced by a radiofrequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier.

Awad El Karim (1994) reported 0.45-0.85%, 1.0-1.6%, 0.18-0.36% and 0.53-0.099% of Calcium, potassium, magnesium and sodium respectively. In the same report United States Pharmacopoeia reported the specifications grade of *Acacia* as arsenic (3ppm), lead (0.001%) and heavy metals (0.004%). The specifications for food grade *Acacia* include arsenic (3mg/kg maximum); heavy metals (0.002% maximum) and lead (5mg/kg maximum) had been cited in the same report. Table (1.3) shows data of cationic composition of *A.senegal* var. *senegal* and *A.seyal* var. *seyal* and *A. nilotica* var. *nilotica* gums.

1.4.8.10 Tannin content

Tannins are, naturally, occurring complex organic compounds of high molecular weight polyphenols. They form colloidal solution with water giving acid reactions. They also precipitate proteins and alkaloids. The astringent nature of tannins is due to the fact that they can preiciptate proteins and renders them resistant to enzymatic attack. When applied on a wound or injury, tannin forms a protective coating, prevent external irritation and thus promote healing.

A study had been done by Zahir (1998, cited by Karamallah, 1999) on raw gums from different *Acacia* species of Sudan-for their taxonomic classification, showed that these

Acacia species could be divided into two main groups. Out of the thirteen gums tested, all but one fell into one group. The species falling in the large group showed presence of tannins in their gums. The tannin content ranged between 0.03 to 1.63%. The only gum that did not show presence of tannin was the gum from *A. senegal,* thus distinguishing itself distinctly and distantly from other *Acacia* gums, Table (1.4). 'This finding was of significant importance when considering gums as food additives. It was established that tannins are anti-nutritional (Karamallah, 1999). Satti (2011) reported the mean values of tannin content for *A.nilotica* var. *nilotica* gum for three seasons (2008, 2009 and 2010) were found 0.08%, 0.08% and 0.1% respectively. The mean values of tannin content reported by Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum were found to be 368.48 and 342.77 ppm.

1.4.8.11Calorific value

The calorific value of a substance, usually a fuel or food, is the amount of heat released during the combustion of a specified amount of it. The calorific value is a characteristic for each substance. It is measured in units of energy per unit of the substance, usually mass, such as: kcal/kg, kJ/kg, J/mol, Btu/m^3. It is determined using bomb calorimeter. Siefeldawla (2018) reported a mean calorific values of 4.04 Kcal/ g for *A.nilotica* var. *tomentosa* gum.

Table 1.3: Cationic composition of some gum samples (ppm). (*A.nilotica* samples as %)

Species	Mg	Ca	K	Na	Zn	Cu	Fe	Mn	Pb	Al	Cr	Ni	References
A.senegal	24000	206000	1600	8400	9.0	32	54	3	0	111	39	3	Anderson *et al.*, 1984[a]
A.senegal	39000	316000	221000	10200	40	66	110	57	11	311	34	12	Anderson *et al*., 1989[b]
A.senegal	38000	256000	237000	940	24	52	128	106	6.0	190	47	10	Anderson *et al*., 1990
A.senegal	1345 – 1987	5387 – 6314	6664 – 7735	3.9 – 12	0.2 – 0.4	1.1 – 1.5	2.5 – 6.9	2.4 – 8.8	< 0.84	4.1 – 11	0.27 – 0.34	< 0.22	Buffo *et al*., 2001[c]
A.senegal	1009	6797	8057.9	792.4	-	23.96	4353	-	-	-	-	-	Omer (2006)
A.senegal	2159.704	7092.2	9459.459	67.1296	20.5125	-	37.0370	-	7.5757	-	-	-	Abdelrahman (2008)
A.senegal	267	6490	261	266	-	-	-	-	-	-	-	-	Younes (2009)
A.seyal	11.7	11200	7900	5.49	620	130	-	750	-	-	-	-	Siddig (2003)[d]
A.seyal	27	7000	101100	9.67	13	51	190	200	-	-	-	-	Siddig (2003)[e]
A.seyal	761	9824	2683	505.5	-	18.82	4339	-	-	-	-	-	Omer (2006)
A.seyal	1229.0424	9417.20	2802.803	111.054	7.8632	-	43.9815	-	7.5757	-	-	-	Abdelrahman (2008)
A.seyal	419	7370	380	195	-	-	-	-	-	-	-	-	Younes (2009)
A.nilotica* var. *nilotica	0.0123-0.0239	0.0918-0.1447	0.0121-0.7615	0.0016-0.0414	1.09-2.54	3.52-6.5	0.0012-0.0031	-	0.0014-0.0082	-	-	-	Satti (2011)
A.nilotica*var. *nilotica	0.0134-0.017	0.048-0.141	0.0411-0.286	0.0018-0.0714	0.0019-0.0036	-	0.0016-0.0048	-	0.0021-0.0081	-	-	-	Alobied (2015)

a,b,d,e cited in Younes (2009), **c** cited in Abdelrahman (2008).

Table1.4: Tannin contents of gums from different *Acacia* species of Sudan (Karamallah, 1999).

Acacia species	Tannin contents (%)
A. senegal var. *Senegal*	0.00
A. seyal var. *seyal*	0.11
A. seyal var. *fistula*	0.09
A. laeta	0.13
A. nilotica subsp. nilotica	0.05
A. nilotica subsp. Tomentosa	0.14
A. drepanolobium	0.03
A. gerrardi	0.16
A. polyacantha	0.16
A. tortillis subsp. Raddiana	1.63
A. mellifera	0.10
A. echrenbergiana	0.17
A. seiberana var *seiberana*	0.08

1.4.8.12 Molecular weight of gum

Molecular weights of *Acacia* gums show wide variations which can be mainly attributed to the method used for the determinations and the heterogeneity of samples (Glicksman and Sand, 1973). These methods include:

(i) Ultra centrifugation (Saverborn, 1944).

(ii) Molecular sieve chromatography (Anderson *et al*, 1966).

(iii) Osmometry (Svvenson *et al*, 1968).

(iv) Intrinsic viscosity measurements (Anderson *et al*, 1983).

(v) Gel filtration (Balabanova and Khristova. 1982).

(vi) Light scattering (Vandevelde and Fenyo. 1985).

The first value reported in literature came from Oakeley (1937) who obtained a molecular weight of 1.91×10^5 and 2.17×10^5 for samples studied in 0.1N and 0.5N sodium chloride solution. Saverborn (1944) using ultra centrifugation method reported values of molecular weight in the range of 2.56×10^5 to 3.26×10^5 g/mol for *A.senegal*. Veis and Eggenberger (1954) using light scattering technique reported average molecule weight value of 0.5×10^5 in 0.02N hydrochloric acid. Mukherjee and Deb (1962) reported value of 0.58×10^5 for

molecular weight of gum Arabic. Mukherjee (1962) reported molecular weight values of 2× 10^5 to 11.6× 10^5 for *Acacia senegal* gum. Anderson *et al*., (1969) estimated the weight average molecular weight for *A. seyal* and the value was 8.5 × 10^5. Fenyo (1988) reported molecular weight value in range of 4.0 × 10^6 to 2.2 × 10^6. Anderson *et al*, (1966) using light scattering techniques reported values of 11.85× 10^5, 3.2× 10^5 and 1× 10^5 for the four values obtained *Acacia senegal* gum and the value of 5.8× 10^5 for the whole gum, using the same method of sodium salt of gum Arabic in molar sodium chloride. Hassan *et al*., (2005) obtained the molecular weight of *A. seyal* from the light scattering measurement using multi angle laser light scattering system. The value of Mw, Mn and Mz were found to be 1.94 x10^6, 1.08x10^5 and 1.11 x10^6 respectively.

Al-Assaf *et al*., (2005) reported a value of 5.99 x 10^5 for the weight average molecular weight using GPC-MALLS of *A. senegal* and a value of 10.4 x 10^5 for *A. seyal* (Abdelrahman 2008) estimated the molecular weight using GPC-MALLS technique. The values of Mw and Mn of *A. seyal* were found to be 15.5 x 10^5 and 5.16 x 10^5. For *A. senegal* were found to be 8.64 x 10^5 and 2.86 x 10^5. He also determined the Mn using osmotic pressure technique and it was found to be 4.7x10^5 and 2.4x10^5 for *A. seyal*, *A. senegal* respectively. Younes (2009) obtained the weight average molecular weights of *A. senegal* and *A. seyal* samples, it was ranged from 8.08 x 10^5 to 1.34 x 10^6 for *A. senegal* and ranged from 6.40 x 10^5 to 1.90 x 10^6 for *A. seyal*.

For *A .nilotica* gum Anderson *et al*., (1969) reported a value of 2.27 x 10^6 g/mol, and a value of 6.74 x 10^5 was reported by Al-Assaf *et al*., (2005). He also reported a value of 1.17 x 10^6 g/mol, 3.86 x 10^5 g/mol, 26 nm and 1.84 for arabinoglactan protein (AGP), arabinoglactan (AG), radius of gyration and polydispersity respectively. Satti (2011) reported a value of 3.4 x 10^6 – 4.82x10^6 g/mol for *A. nilotica* var. *nilotica* gum. Siefeldawla (2018) reported values of the weight average molecular weight for *A.nilotica* var. *tomentosa* gum ranged from 5.03 x10^6 to 8.9 x10^6 g/mol.

1.4.8.12.1 Fractionation of *Acacia* gum

Vandevelde and Fenyo (1985) showed that gum Arabic consists of two fractions: one which constitutes the majority of the gum, has little, protein, whilst the second is a glycoprotein, subsequently, Randall *et al*, (1989) fractionated the gum using hydrophobic chromatography. They isolated a third fraction in addition to the fractions isolated by Vandevelde *et al*, (1985). Analysis of the Fractions showed that fraction 1, which does not adsorb to phenyl sepharose

and passes straight through the column, does not contribute any significant emulsifying activity (Arabinogalactan, AG). Fraction 2 (Arabinogalactan-protein complex, AGP) and fraction 3 represent only 12.4g per kg of the total gum (glycoprotein, GP) which is suspected to be the most important contributor to the gum emulsifying properties. Underwood *et al*, (1994) fractionated gum Arabic using hydrophobic column chromatography into eight fractions by dissolving 25g of the gum into 4.2 M NaCl; insolubles were removed by centrifugation. The sample was then cooled to 5 $^{\circ}$C then loaded onto a 450 ml phenyl Sepharose CL4B Pharmacia column (85 X 2.6 cm) at 5 $^{\circ}$C. The column was eluted with 4.2 M NaCl as an eluant, then water, 1M NaOH and 1M HCl, fractions corresponding to each component were pooled, dialyzed against distilled water , freeze-dried and weighed.

Osman *et al*, (1994) fractionated *A. senegal* using hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC). They reported that five fractions were obtained. Fraction 1 showed no affinity to DEAE-cellulose and was eluted by phosphate buffer only. Fractions 2, 3, 4 and 5 were eluted by stepwise increase ionic strength of the phosphate buffer using NaCl, from 0.02 to 0.2 mol/dm^3 in increment of 0.04 mol/dm^3.

Hassan *et al.*, (2005) fractionated *A. seyal* using GPC technique and *A. senegal* was used as control. They reported that two fractions were obtained for *A. seyal* and *A. senegal*. High molecular weight fraction with an average molecular mass of 5.3 x 10^6 and 2.2 x 10^6 were obtained for *A. seyal* and *A. senegal* respectively, and a low molecular weight fraction with an average molecular mass of 1.0 x 10^6 and 3 x 10^5 were obtained for *A. seyal* and *A. senegal* respectively. They concluded that the protein distribution in *A. seyal* is different from that in *A. senegal*, whereas it is exclusively associated with the high molecular weight component (AGP) in *A. senegal*. For *A. seyal* the protein is distributed between the molecular weight fractions and the lower ~1 x 10^5 molecular weight component.

Renard *et al*, (2006) used hydrophobic interaction chromatography (HIC) technique to fractionate *Acacia* gum using phenyl Spharose Cl-4B (Pharmacia, Uppsala, Sweden). They reported that the bulk of the gum (88.4 wt % of the total) was shown to be comprised by the so-called arabinogalactan (AG) fraction with weight-average molecular weight of 2.79X10^5 g.mol^{-1} from light scattering measurements and low in protein (0.35 wt %). The second major fraction (10.4 wt % of the total) was identified as an arabinogalactan-protein complex (AGP) with a molecular weight of 1.45 X 10^6 gmol^{-1} and contained a greater proportion of protein (11.8 wt %). The third minor fraction (1.24 wt %) consisted of one, or possibly two, glycoprotein's (GP). One of the GP had a molecular weight of 2.5 X 10^5 g.mol^{-1} and the highest protein content (47.3 wt %), Table (1.5).

Vandevelde and Fenyo (1985) and Duvallet *et al*.,(1989) showed that enzyme degradation of gum decrease the weight average molecular weight (Mw) from 7.2 x 10^5 to 1.8 x 10^5 whereas number average molecular weight (Mn) remain unchanged at value of 1.9x10^5, and that Mw approached Mn value. They suggested then that Mn is more fundamental property than Mw and hence Mn could be considered as an intrinsic property of gum. Connolly *et al*., (1987, 1988) carried out enzymatic degradation studies on the whole gum of *A. senegal* using pronaese, and found that the higher molecular mass components were degraded to a single component of lower molecular mass. They argued that their results were consistent with the "wattle blossom" model for AGPs proposed by Fincher *et a1* (1983), and suggested that the molecules consisted of several polysaccharide blocks of molecular mass about 2 x $l0^5$ linked to a common protein core.

Table 1.5: Molecular parameter of *Acacia* gum and its molecular fractions (Renard *et al*, 2006)

[a] Fraction resulting from two HIC separations. [b] Fractions resulting from one HIC separation. [c] By HPSEC-MALLS. [d] Rg value calculated using the Flory-Fox equation $[\eta]Mw) 6^{3/2}\theta Rg^{3}$ where θ is the Flory viscosity constant (~2.2 $X10^{23}$). [e.] By differential viscosimetry. [f.] Hydrodynamic radius determined by quasi-elastic light scattering at three angles (30, 90, and 150°) in 0.05 M $NaNO_3$ buffer

	Total gum		Fraction I[a]	Fraction II[b]	Fraction III[c]		
	First population (11.3 – 13.3 ml)	Second population (14.3 -17.1ml)	(13.8 -17.2ml)	(11.5-5.6ml)	First population (16.3-17.3ml)	Second population (14.2-15.6ml)	Third population (16.3-17.3ml)
C area	11.5	86.5	100	97.4	48.5	31.0	20.5
dn/dc	0.147	-	0.145	0.171	-	0.153	-
Mw $g.mol^{-1}$[c]	$2.69 X 10^6$	3.06	$2.86 X 10^5$	$1.86 X 10^5$	$2.67 X 10^6$	$7.76X 10^5$	$2.95X 10^5$
Mn $g.mol^{-1}$[c]	$2.30 X 10^6$	2.44	$2.23 X 10^5$	$1.40 X 10^5$	$2.36 X 10^6$	$7.46X 10^5$	$2.92X 10^5$
Mw/Mn	1.17	1.25	1.28	1.33	1.13	1.04	1.01
Rg nm	35.5	11.8[d]	11.3[d]	30	41.3	25.3	19.5
[η] $ml\ g^{-1}$[e]	80.2	17.3	16.2	70.7	102.6	64.4	29.8
R_h nm (QELS)[f]	10.7		9.1	34.4		16.1	

Al-Assaf *et al*., (2005) fractionated *A. nilotica* using gel permeation Chromatography (GPC). The elution profile indicated by a detector measuring refractive index and light scattering detector diode at 90°, showed two peaks. One major component with very high molecular weight but present in relatively low concentration, and another although, of lower molecular weight is present in the greater concentration. The high molecular weight component contains the majority of the protein, but represents a small part of the gum only. This has been identified as an arabinogalactan protein complex (AGP). The main component, which has a lower amount of protein but constitutes the majority of the gum, has been designated an arabinogalactan (AG). Abdelrahman (2008) estimated the molecular weight using GPC-MALLS technique. The values of Mw and Mn of *A. seyal* were found to be 15.5 x 10^5 and 5.16 x 10^5. For *A. senegal* were found to be 8.64 x 10^5 and 2.86 x 10^5. He also determined the Mn using osmotic pressure technique and it was found to be 4.7x10^5 and 2.4x10^5 for *A. seyal*, *A. senegal* respectively. Younes (2009) obtained the weight average molecular weights of *A. senegal* and *A. seyal* samples, it was ranged from 8.08 x 10^5 to 1.34 x 10^6 for *A. senegal* and ranged from 6.40 x 10^5 to 1.90 x 10^6 for *A. seyal*.

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Satti, (2011) fractionated *A. nilotica* var. *nilotica* using gel permeation chromatography (GPC). Fraction one, which constitutes the majority of the gum arabinoglactan component (AG) with weight average molecular weight 2.97 x 10^6 .The elution profile of the hydrophilic fraction 1 contained slightly less AGP component (25.25%).

Fraction two is arabinoglactan protein (AGP) with weight average molecular weight 9.37 x 10^6. The elution profile of the hydrophobic fraction 2 showed that it is essentially the GP fraction table (1.6). (Satti, 2011) showed that enzyme degradation of *A.nilotica* var. *nilotica* gum using gel permeation chromatography(GPC) elution profile showed The peak 1, of high molar mass, decreases in concentration after 48h. In contrast, the peak of lower molecular weight (peak 2) increases. The UV elution profile shows that the treatment with protease gives rise to a gradual decrease in the intensity of peak 1 and a sudden increase in the intensities of peaks 2. Consequently, the polydispersity index is lower for protaese treated gum (2.2) than for native gum (2.5) but the molecular system is still very complex, and the molecular mass decrease from 3.59 x 10^6 to 2.8 x 10^6.

Table1.6: GPC-MALLS analysis of the hydrophilic and hydrophobic fractions derived from *A. nilotica* var. *nilotica* control gum (Satti, 2011).

Sample	**Processing**	**Molecular weight (Mw, g/mol)**	**Recovery (%)**	**Polydispersity (Mw/Mn)**	**Rg (nm)**
Control gum	Total	3.59×10^6	116.06	2.348 ± 0.074	39.7
	First peak (AGP)	8.35×10^6	29.09	1.126 ± 0.043	44.9
	Second peak (AG+GP)	1.63×10^6	70.96	1.415 ± 0.030	25.8
Hydrophilic fraction (fraction 1)	Total	2.97×10^6	120.63	1.552 ± 0.015	27.8
	First peak (AGP)	6.50×10^6	30.46	1.061 ± 0.008	32.2
	Second peak (AG+GP)	1.78×10^6	90.24	1.146 ± 0.011	21.1
Hydrophobic Fraction (fraction 2)	Total	2.43×10^5	146.68	5.093 ± 1.587	54.5
	First peak (AGP)	9.37×10^6	0.97	1.180 ± 0.273	58.5
	Second peak (AG+GP)	1.81×10^5	144.84	3.81 ± 1.163	53.1

Table 1.7: Analytical data for *A. nilotica* gum

Species	Moisture %	Ash %	pH	Nitrogen %	Protein %	Specific rotation (degrees)	Intrin. Viscosity ml/g	Equivalent weight	Uronic acid %	Glucuronic acid %	Glactose %	Arabinose %	Rhamnose %	Authors
A. nilotica* var. *nilotica	-	-	-	0.02	-	+107	-	1890	9.3	-	42	46	-	Anderson (1966)
A. nilotica* var. *nilotica	-	2.48	-	0.02	-	+108	9.5	1890	9.0	3.0	44	46	0.4	Anderson (1977)
***A. nilotica* Indian gum**	-	-	-	-	1.92	-	-	-	11.3	-	30.2	55.1	2.6	Kapoor(1991)
A. nilotica* var. *nilotica	6.1	0.03	4.1	0.06	0.37	+97.66	-	-	-	-	-	42	1.8	Karamalla (1999)
A. nilotica* var. *adstringens	5.6	0.06	3.75	0.06	0.37	+75.16	-	-	-	-	-	-	-	Karamalla (1999)
***A. nilotica* Nigerian gum**	-	-	-	-	47	+21	35	-	21	-	18	25	6	AlAssaf (2005)
A. nilotica* var. *nilotica	10.84	1.90	5.08	0.025	0.163	+100	10.13	1899.87	10.21	-	17.43	41.20	10.68	Satti (2011)
A. nilotica* var. *nilotica	10.87	1.86	5.1	0.023	0.151	+92.6	10.10	1866.76	10.42	-	16.34	45.90	10.33	Alobied (2015)
A. nilotica* var. *tomentosa	11.95	1.94	5.13	0.05	0.28	+91.8	10.16	2182.86	8.88	-	-	-	-	Siefeldawla (2018)

Table1.8: Physicochemical properties *A. nilotica* gum exudates (Nigerian gum) (Yusuf, 2011)

Physicochemical parameter	*A. Nilotica* gum
Moisture (%)	15.60
Solubility (%)	38
Melting temperature (C^o)	300-320
Relative density of 20% solution (35 C^o)	1.32
Relative viscosity of 1% solution (30 C^o)	24.80
pH (25% solution)	4.50
Ash (%)	3.54
Tannin	0
Nitrogen (% W/W)	0.40
Protein (Nx6.6)	2.71
Total soluble fiber (%)	78.15
Calcium (g/100g)	0.70
Magnesium (g/100g)	0.30
Iron (g/100g)	0.0004
Sodium (g/100g)	0.016
Potassium (g/100g)	0.78

1.4.9 Emulsification properties of *Acacia nilotica* var. *adstringens* gum

1.4.9.1 Emulsions

An emulsion consists of two immiscible liquids, with one of the liquids being dispersed as small spherical droplets in the other liquid (Dalgleish**,** 2001). In the food industry, the two immiscible liquids are usually oil and water. The mean diameter of the droplets in emulsified food products typically falls somewhere in the range of 0.1 to 100 μm.

1.4.9.2Types of Emulsion

Emulsions can be, conveniently, classified according to the relative spatial distribution of the different phases. An emulsion consisting of oil droplets dispersed in an aqueous phase is referred to as oil in water (O/W) emulsion, such as milk, cream, ice-cream, dressings, mayonnaise, beverages, soups, dips and sauces. An emulsion that consists of water droplets dispersed in an oil phase is referred to as water in oil (W/O) emulsion, such as butter, margarine and some spreads. The material that makes up the droplets is usually referred to as the dispersed phase, discontinuous phase or internal phase, whereas the material that makes up the surrounding liquid is referred to as the dispersing phase, continuous phase or external phase. It is also possible to create various types of multiple emulsions**,** such as oil in water in oil (O/W/O), water in oil in-water (W/O/W), oil in water in water (O/W/W). The process of converting bulk oil and bulk water into an emulsion, or of reducing the size of the droplets in an already existing emulsion, is known as homogenization. Homogenization is usually achieved by applying intense mechanical agitation to a liquid mixture using a mechanical device known as a homogenizer, such as a high shear mixer, a high pressure valve homogenizer, a colloid mill, a microfluidizer or an ultrasonic homogenizer (Mc Clements, 2007, Hasenhuettl *et al*., 2008). Macroemulsions are inherently thermodynamically unstable systems because the contact between oil and water molecules is unfavorable, and so they will always breakdown over time.

The preparation of emulsions that are kinetically stable over a time period requires the incorporation of substances known as stabilizers or emulsifying agent (Dickinson, 1992).

1.4.9.3 Emulsifying agents

Emulsifiers (emulsifying agents) are substances with substantial surface activity at the oil-water interface, and have the ability to expedite the formation and stabilization of fine droplets during and after emulsification (Dickinson, 2003; 2004). Emulsifying agents or

stabilizers can be classified according to their mode of operation as either emulsifiers or texture modifiers. An emulsifier is a surface active substance that adsorbs to the surface of emulsion droplets to form a protective coating that prevents the droplets from aggregating with one another, such as certain proteins, polysaccharides, phospholipids and small molecule surfactants (Whitehurst, 2004). An emulsifier also reduces the interfacial tension and therefore facilitates the disruption of emulsion droplets during homogenization, which aids in the formation of emulsions containing smaller droplets (Dalgleish**,** 2001). A texture modifier is a substance that either increases the viscosity of the continuous phase (thickening agent) or forms a gel network within the continuous phase (gelling agent), thereby slowing down the movement of droplets due to gravity or Brownian motion. Many types of polysaccharide and protein ingredients can be used as thickening or gelling agents in food emulsions, including gum arabic, starch, modified starch, cellulose, modified cellulose, pectin, alginate, carrageenan, gelatin, whey protein, caseinate, soy protein and egg protein (Hasenhuettl *et al*., 2008).

1.4.9.4 Instability Mechanisms

The term emulsion stability refers to the ability of an emulsion to resist changes in its physicochemical properties over time. Food emulsions may become unstable due to a variety of different physicochemical mechanisms (Dickinson, 1992, Dalgleish**,** 2001, Mc Clements, 2007), including gravitational separation (creaming and sedimentation), flocculation, coalescence, and Ostwald ripening and phase inversion (Figure 1.12).

1- Gravitational separation is the process whereby droplets move upward (creaming) because they have a lower density than the surrounding liquid, or downwards (sedimentation) because they have a higher density than the surrounding liquid.

2- Flocculation is the process whereby two or more droplets stick together to form an aggregate in which each of the initial droplets retains its individual integrity.

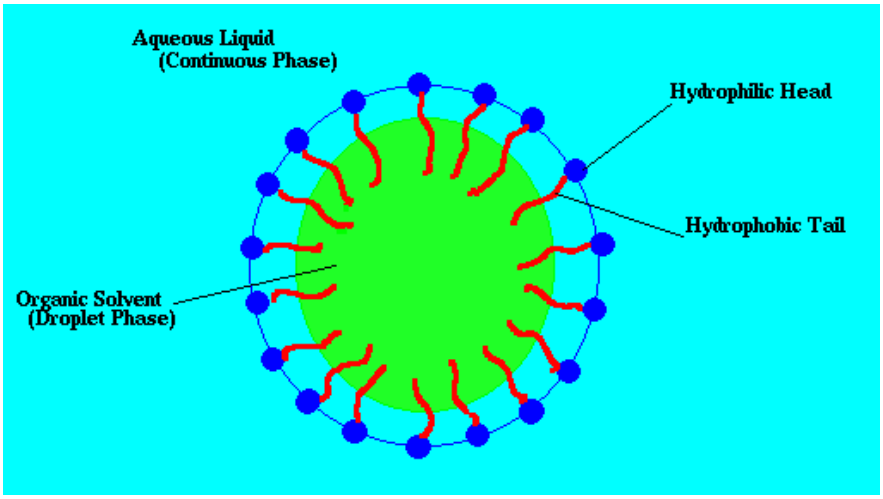


Figure 1.11: Schematic diagram depicted emulsifying agent

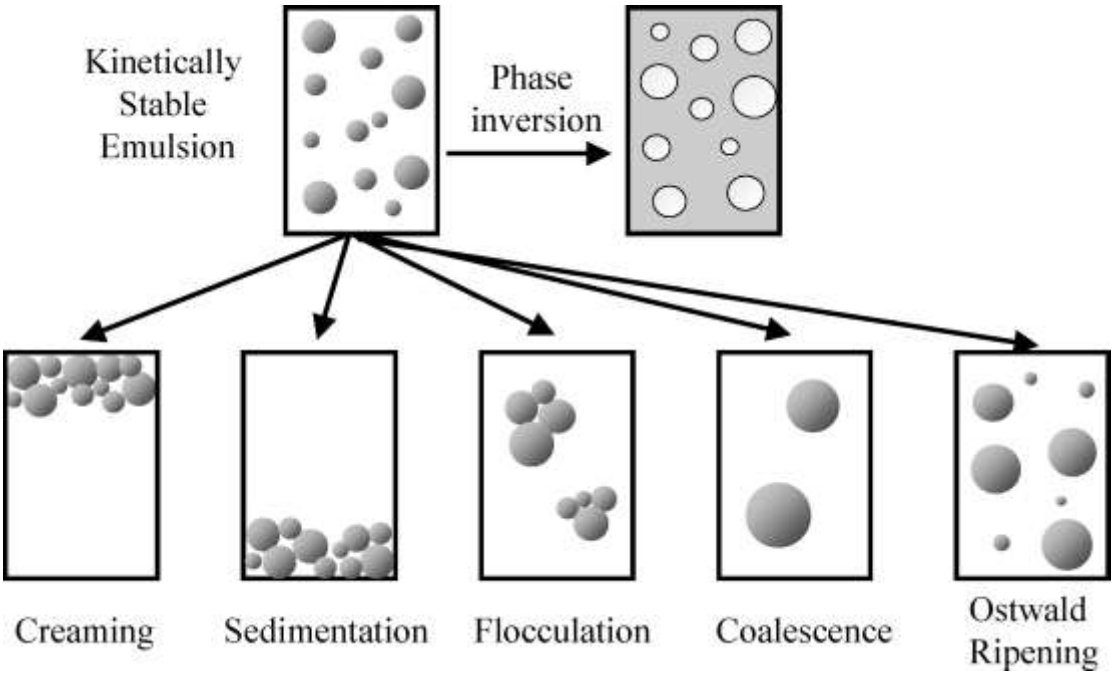


Figure 1.12: Schematic diagram of most common instability mechanisms.

3- Coalescence is the process whereby two or more droplets merge together to form a single larger droplet.

4- Ostwald ripening is the process whereby larger droplets grow at the expense of smaller droplets due to mass transport of dispersed phase material through the continuous phase.

5- Phase inversion is the process whereby an oil-in-water emulsion changes to a water-in-oil emulsion, or vice versa (Mc Clements, 2007).

Therefore there are two fundamental mechanisms that affect dispersion stability of colloidal:

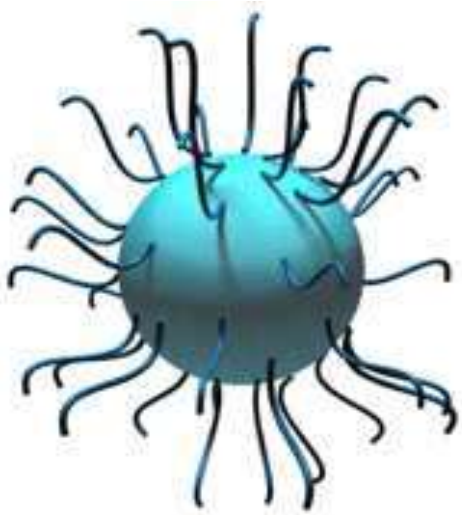
1. Steric repulsion - this involves polymers added to the system adsorbing onto the particle surface and preventing the particle surfaces coming into close contact. If enough polymers adsorbs, the thickness of the coating is sufficient to keep particles separated by steric repulsions between the polymer layers, and at those separations the Van der Waals forces are too weak to cause the particles to adhere.

2. Electrostatic or charge stabilization - this is the effect on particle interaction due to the distribution of charged species in the system.

Each mechanism has its benefits for particular systems. Steric stabilization is simple, requiring just the addition of a suitable polymer.

However it can be difficult to, subsequently, flocculate the system if this is required, and the polymer can be expensive and in some cases the used polymer is undesirable e.g. when a ceramic slip is cast and sintered, the polymer has to be 'burnt out'. This causes shrinkage and can lead to defects (Salford, 2008).

El-Kheir, Abu El-Gasim and Baker, (2008) reported that, the stability of gum Arabic emulsions was significantly affected by the types of oil used and the source of the gum. The adsorbed gum Arabic surface layer is able to prevent droplet flocculation and coalescence through both electrostatic and steric repulsive forces (Funami *et al*., 2008; Li *et al*., 2009).



Steric stabilization

Electrostatic stabilization

1.4.9.5 Droplet Characteristics

The physicochemical properties of emulsions are strongly influenced by the characteristics of the droplets that they contain, such as their concentration, size, charge, interfacial properties and interactions (Mc Clements, 2005, Hasenhuettl, 2008).

1.4.9.5.1 Droplet Concentration

The concentration of droplets in an emulsion influences its texture, stability, appearance, and nutritional quality (Mc Clements, 2005). Droplet concentration is usually characterized in terms of the dispersed phase volume fraction (∅), which is equal to the volume of emulsion droplets (V_D) divided by the total volume of emulsion (V_E): $\emptyset = V_D/V_E$. Practically, it is often more convenient to express the droplet concentration of an emulsion in terms of the dispersed phase mass fraction ($\emptyset_m$), which is equal to the mass of emulsion droplets (m_D) divided by the total mass of emulsion (m_E): $\emptyset_m = m_D/m_E$.

The relationship between $\emptyset_m$ and φ is given by the following Equations:

$$\emptyset_m = \emptyset\left(\emptyset + (1-\emptyset)\,{}^{\rho_1}/_{\rho_2}\right)^{-1} \quad \text{..... (1.5)}$$

$$\emptyset = \emptyset_m\left(\emptyset_m + (1-\emptyset_m)\,{}^{\rho_2}/_{\rho_1}\right)^{-1} \quad \text{..... (1.6)}$$

Where, $\rho 1$ and $\rho 2$ are the densities of the continuous and dispersed phases, respectively. When the densities of the two phases are equal, the mass fraction is equivalent to the volume fraction. The droplet concentration may also be represented as either a dispersed phase volume percentage ($\emptyset\% = 100 \times \emptyset$) or disperse phase mass percentage ($\emptyset_m\% = 100 \times \emptyset_m$).

1.4.9.5.2 Droplet Size

The size of the droplets in an emulsion has a strong impact on its stability, optical properties, and its rheology (Dalgleish, 2001). It is therefore particularly important to be able to reliably measure and accurately specify the size of the droplets present within an emulsion.

When all the droplets in an emulsion have the same size, the emulsion is referred to as monodisperse, and a single number, either the droplet radius or droplet diameter, can be used to characterize the droplet size. In practice, food emulsions contain a range of different droplet sizes, and are therefore referred to as being polydisperse.

A polydisperse emulsion is characterized by its particle size distribution, which -defines the concentration of droplets in different size classes. The huge number of droplets in most food emulsions means that the particle size distribution can be considered to be continuous.

When constructing or interpreting a particle size distribution (PSD), the particle concentration is usually presented as either the volume percent (Volume %) or number percent (Number %) of droplets, whereas the particle size is usually presented as either the mid-point particle radius or the mid-point particle diameter.

It is important to realize that a number of different mean particles sizes can be derived from a full particle size distribution and each mean size can have a different magnitude and physical meaning. In general, the mean (x_{ab}) and relative standard deviation (c_b) of a distribution can be defined by the following equations (Walstra, 2003):

$$x_{ab} = \left(\frac{\sum_{i=1}^{N} n_i x_i^a}{\sum_{i=1}^{N} n_i x_i^b}\right)^{1/(a-b)} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1.7)$$

$$c_b = \left(\frac{\left(\sum_{i=1}^{N} n_i x_i^b\right)\left(\sum_{i=1}^{N} n_i x_i^{b+2}\right)}{\left(\sum_{i=1}^{N} n_i x_i^{b+1}\right)^2} - 1\right)^{1/2} \quad \ldots\ldots (1.8)$$

Where, a and b are integers (usually between 0 and 4), n_i is the number of droplets with size x_i, and N is the total number of size categories present.

The three most commonly used mean particle size values are:

The number weighted mean diameter $(d_{10} = \sum n_i d_i / \sum n_i)$ …… (1.9)

The surface weighted mean diameter $(d_{32} = \sum n_i d_i^3 / \sum n_i\, d_i^2)$…… (1.10)

The volume weighted mean diameter$(d_{43} = \sum n_i d_i^4 / \sum n_i\, d_i^3)$. ….. (1.11)

Generally, the volume weighted mean diameter is more sensitive to the presence of large particles than the number weighted mean diameter. Appreciable differences between the values of d_{10}, d_{32} and d_{43} generally indicate that the particle size distribution is broad or multimodal. One must therefore be very careful when interpreting or reporting particle size data to identify which mean particle size value is being used (Satti, 2011).

1.4.9.5.3 Droplet Charge

The droplets in most emulsions have an electrical charge because of adsorption of molecules to their surface that are ionized or ionizable, such as proteins, polysaccharides, ionic surfactants, phospholipids and some small ions. The electrical characteristics of a droplet surface depend on the type and concentration of ionized charge species present at the surface, as well as the ionic composition and physical properties of the surrounding liquid. The charge on an emulsion droplet is important because it determines the nature of its interactions with other charged species or its behavior in the presence of an electrical field. The droplets in many emulsions are prevented from aggregating by using ionic emulsifiers that adsorb to

their surface and prevent them from coming close together because of electrostatic repulsion (Dickinson, 1992, Dalgleish, 2001, Mc Clements, 2005).

The electrical characteristics of a droplet are usually characterized in terms of its surface electrical potential (ψ), surface charge density (σ) and zeta potential (ζ). The surface charge density is the amount of electrical charge per unit surface area. The surface electrical potential is the amount of energy required to increase the surface charge density from zero to σ, by bringing charges from an infinite distance to the surface through the surrounding medium. The zeta potential (ζ) is the electrical potential at the shear plane, which is defined as the distance away from the droplet surface below which the counter ions remain strongly attached to the droplet when it moves in an electrical field. Practically, the droplet charges are usually characterized in terms of ζ (Hunter, 1986).

1.4.9.5.4 Interfacial Properties

The droplet interface consists of a narrow region (~1 to 50 nm thick) that surrounds each emulsion droplet, and contains a mixture of oil, water, and emulsifier molecules. The interfacial region only makes up a significant fraction of the total volume of an emulsion when the droplet size is less than about 1 μm. The properties of the interfacial region are determined by the type, concentration, complexation, competitive adsorption and layer by layer formation. The electrical charge on the droplet interface influences its interaction with other charged molecules, as well as its stability to aggregation. The thickness and rheology of the interfacial region influences the stability of emulsions to gravitational separation, coalescence and flocculation, and determines the rate at which molecules leave or enter the droplets (Dickinson, 2003, Mc Clements, 2005).

1.4.9.5.5 Colloidal Interactions

Colloidal interactions govern whether emulsion droplets aggregate or remain as separate entities, as well as determine the characteristics of any aggregates formed, such as their size, shape, porosity and deformability (Friberg *et al*., 2004, Mc Clements, 2005). The interactions between two emulsion droplets can be described in terms of an interaction potential. The interaction potential is the energy required to bring two emulsion droplets from an infinite distance apart to a surface to surface separation. Generally, droplets tend to aggregate when attractive interactions dominate, but remain as individual entities when repulsive interactions dominate (Mc Clements, 2005).

1.4.9.6 Testing the emulsion stability

The emulsion stability can be tested by following the growth of the droplet size of the dispersed medium with time by one of the different physical methods. These physical methods are all optical methods. They depend on the intensity of light scattered by particles and molecules when a beam of light is passing through the solution of the sample being measured. One of the most reliable methods of testing the emulsification properties of a certain surfactants is to measure directly the growth of the sizes of the droplets together with the span and the specific surface area with time using a mastersizer laser diffractometer device. Another method can also be used is to follow the turbidity of the solution with time by using a turbidity meter device. The turbidometric technique is less informative than the direct particle size analysis but the technique is still remaining a useful qualitative technique for comparing the relative emulsifying properties of different surfactants (Sadar, 1998).

The stability of the emulsion can also be determined from a physical parameter called the stability index which is defined as follows:

$$Stability\ index\ = \frac{First\ reading\ at\ zero\ time}{reading\ at\ (x)\ time}$$

If the stability index is unity or close to unity then the emulsion is considered as stable (i.e. no change or slightly change with time), otherwise it is less stable.

1.4.9.7 Particle size determination

Particle sizing instruments that utilize static light scattering, also called laser diffraction are based on the principle that the scattering pattern (intensity of scattered light versus scattering angle) produced when a laser beam is directed through a dilute emulsion depends on the particle size distribution. Instruments come with software that contains a mathematical model, usually the "Mie theory", that can predict the scattering pattern of an emulsion from the characteristics of the particles that it contains such as refractive index ratio, absorption coefficient, and diameter. The software finds the particle size distribution that gives the best fit between the measured scattering pattern and the theoretically predicted one, and then reports the data as a table or plot of particle concentration such as number or volume versus particle size such as diameter or radius. Commercial static light scattering instruments are capable of determining particle diameters within the range of about 100 nm to 1000 μm.

These instruments normally require that the droplet concentration be relatively low, < 0.1 wt% so as to be able to pass a light beam through and to avoid multiple scattering effects. Consequently, many food emulsions need to be diluted considerably prior to analysis (Mc Clements, 2007).

The following parameters have been calculated for each emulsion:

*Span% = value for the broadness of the distribution, it is determined by:

Span %= d (0.9)-d (0.1) /d (0.5)

Where:

d (0.9) = the particle diameter, which covers 90% of the particles.

d (0.1) = the particle diameter, which covers 10% of the particles.

d (0.5)= particle diameter which covers 50% of the particles.

*Surface area

* And the particle size distribution of each solution was obtained.

1.4.9.8 Emulsification properties of gums

Gum Arabic has been used as emulsifier and emulsion stabilizer since ancient times. It is used as an emulsifier in many flavors used in soft drinks, such as fruit flavors, cola, and root beer.

The main role of an emulsifier is to adsorb at the surface of freshly formed fine droplets, and prevent them from coalescing with their neighbors to form larger droplets. For a fixed rate of energy dissipation during emulsification, the final droplet size distribution is determined by the time taken for the interface to be covered with emulsifiers, as compared with the average time interval between droplet collisions. When emulsifiers adsorb too slowly, or are present at too low concentration, most of the individual droplets formed during the intense energy dissipation of emulsification are not retained in the final emulsion (Dickinson, 2009b).

Gums are effective primarily in (O/W) emulsions, and the concentration must be high enough to have a marked thickening effect on the aqueous phase. The function of emulsifier or stabilizer is essentially to increase the viscosity of the aqueous phase by thickening it so that it approximates or slightly exceeds that of the oil. In this way the tendency of the dispersed phase to slip or coalesce is minimized, and the emulsion is, so to speak, stabilized.

During the process of making a mixture of two immiscible substances, minimal contact between the oily phase and water can initially be achieved by the formation of small spherical droplets through the input of work and addition of emulsifier, facilitating the formation of

these small particles by reducing interfacial tension. In fact, the aim of emulsification such as (O/W) is to produce a small size of droplets of the dispersed phase (oil) and the size of the droplets should not be increased during emulsion storage. This means that emulsions have to be stable for a certain period of time. Gum Arabic forms a protective layer around the oil droplets that prevents the droplets from aggregating such as flocculating and coalescing. It also reduces the oil water interfacial tension, thereby facilitating the disruption of emulsion droplets during homogenization (Dickenson, 2003).

The ability to form stable emulsions over a wide range of pH and in the presence of electrolytes made this biopolymer (gum Arabic) an appropriate natural emulsifier which has been intensively used in food industries, particularly in the beverage sector. Considering the complexity of gum Arabic molecular structure and its relation to the emulsifying function has encouraged many researchers to conduct studies in the hope of finding a suitable substitute for gum Arabic. Degen, Vidoni and Rehage, (2012), observed that, the presence of both hydrophilic sugar residues and hydrophobic amino acids are the main components which give it the ability to adsorb at oil-water interfaces.

In emulsions, the hydrophobic polypeptide chains are adsorbed at the oil - water interface, while the hydrophilic polysaccharide protrudes into the aqueous phase to form the dense layer (Buffo, Reineccius and Oehlert, 2001). Figure (1.13) shows the schematic illustration of the stabilization of the oil droplets by gum arabic molecule. Randall *et al*., (1988, 1989) showed that *A. senegal* was a polydisperse mixture of proteinous components composed by three minor components. Two of them do not adsorb at the interface and there is also a third one that it was the arabinogalactan protein (AGP) component which is responsible for the emulsification properties.

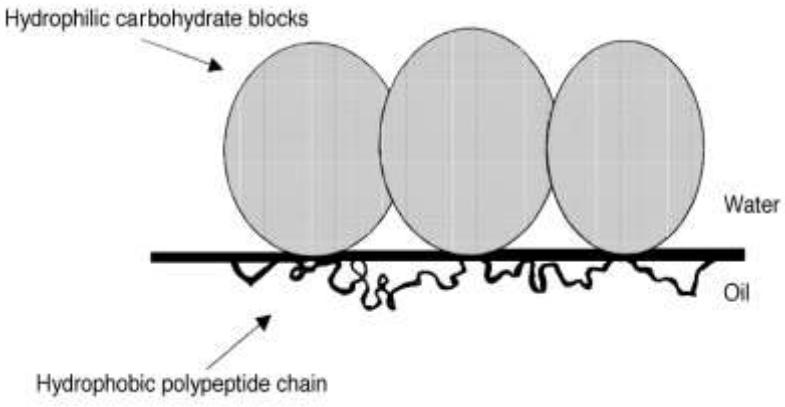


Figure 1.13: Schematic illustration of the stabilization of the oil droplets by gum

Several polysaccharide units are linked to a common protein core in this high molecular weight component (M_W ~ $2.5x10^6$ Da). The most accepted model for the emulsifying fraction of gum 58tabil is the 'wattle blossom (Osman *et al*., 1993). Still other models, which involve electrostatic contribution as well as steric forces, have also been proposed (Ray *et al*., 1995; Islam *et al*., 1997). On the other hand, there does appear to be good correlation between emulsion stability and gum 58tabil average molecular weight. It was observed (Dickenson, 2003) that the 10% fraction of a gum 58tabil sample corresponding to the highest molecular weight (0.38% N), as separated by GPC could produce a more stable emulsion than the remaining 90% fraction (0.35% N). Additionally, in separate experiments, when the average molecular mass of gum 58tabil sample (~0.35% N) was gradually reduced from $3.1x10^5$ to $2.2x10^5$ Da by controlled irradiative degradation, the resulting emulsion stability was correspondingly reduced (Dickenson, 2003). The smaller arabinogalactan units, containing some protein associate to form larger molecular mass AGP aggregates. In all aspects this specially matured gum is chemically and molecularly identical to the base gum, but because of the difference in distribution of the proteinaceous components, the physical and functional performance is greatly enhanced.

Erni *et al*., (2007) earlier studied the elastic stabiliz of the adsorption layer of gum Arabic and suggested that the elastic 58tabiliz plays a significant role in the stabilization of oil/water emulsions. Gum Arabic is also able to form thick viscoelastic films, by adsorbing onto the oil-water interface, thereby reducing the interfacial tension between oil and water thus performing as an emulsifying agent (Tan, 2004).

1.4.10 Rheological properties of *Acacia nilotica* var. *adstringes* gum

Rheology is the science of deformation and flow. It reveals information about the flow behaviour of liquids and the deformation behaviour of solids. All forms of shear behaviour, which can be described rheologically in a scientific way, can be looked upon as lying in between two extremes: the flow of ideal viscous liquids on one hand and the deformation of ideal elastic solids on the other.

1.4.10.1Definitions and principles of some rheological terms

- **Shear stress**: $\tau = F/A$ where F is the (shear) force and A is the shear area.
- **Shear deformation (or shear strain)**: $\gamma = s/h$, where s is the deflection and h is the distance between the plates.

- **Shear modulus:** $G = \tau / \gamma$, it reveals information about the rigidity of a material. Materials with comparably strong intermolecular cohesive forces show a higher internal rigidity and therefore also a higher value of G.
- **Shear rate**: $\dot{\gamma} = v/h$, where v is the velocity and h is the gap between the plates. (The following terms are also used: rate of deformation, strain rate, shear gradient or velocity gradient).

1. 4.10.2 Rheological classification of fluids

In classical mechanics, the distinction between liquids and solids was very clear and separate physical laws existed to describe the behavior of solids (Hooke's law) and liquids (Newton's law). However, a variety of products (such as food) exist that exhibit intermediate behavior which needs to be well characterized.

Fluids are initially classified as having Newtonian Fig (1.14) or Non-Newtonian behavior Fig (1.15) , depending on whether they can be described by Newton's law of viscosity or not.

Non-Newtonian fluids are also classified as time-dependent or time-independent. Fluids in which rheological behavior depends only on the shear stress (at constant temperature) are considered time-independent. Time-dependent fluids are those in which the viscosity depends, not only on the shear stress, but also on the length of time the stress has been applied to fluid. There are fluids that present both viscous and elastic behavior; they are called viscoelastic fluids.

Classification of flowing fluids can be done by means of viscometric functions. For Newtonian fluids, the viscosity function is constant, and the viscosity (Newtonian viscosity) is independent of shear strain rate and time ($\eta(\dot{\gamma}) = \eta$ = constant).

In non-Newtonian fluids, the viscosity function depends on shear strain rate, and the apparent viscosity is defined as:

$$\eta_a = \delta_{12} / \dot{\gamma} = \eta(\dot{\gamma})$$

Fluids can be classified according to the following scheme:

A) Newtonian flow.

B) Non-Newtonian flow.

1- Time independent:

a) Plastic fluids.

b) Pseudoplastic fluids (shear- thinning).

c) Dilatent fluids (shear –thickening).

2- Time dependent:

a) Thixotropic fluids.

b) Anti-thixotropic or rheopectic fluids.

c) Viscoelastic fluids.

1.4.10.2.1 Newtonian or Bingham Flow

Eugene Bingham, a colloid chemist, first coined the term “Rheology.” He also showed that for many real fluids a critical level of stress must be attained in order to initiate flow. Below this critical stress, τy, the material behaves as a solid, absorbing the stress energy without flowing. Once the threshold of critical stress has been reached, the material yields to flow, hence the term, yield stress. The yield stress is the reason, why you need to shake or tap a bottle to make the ketchup flow. Materials which exhibit Newtonian flow beyond the yield bear the name Bingham Fluids.

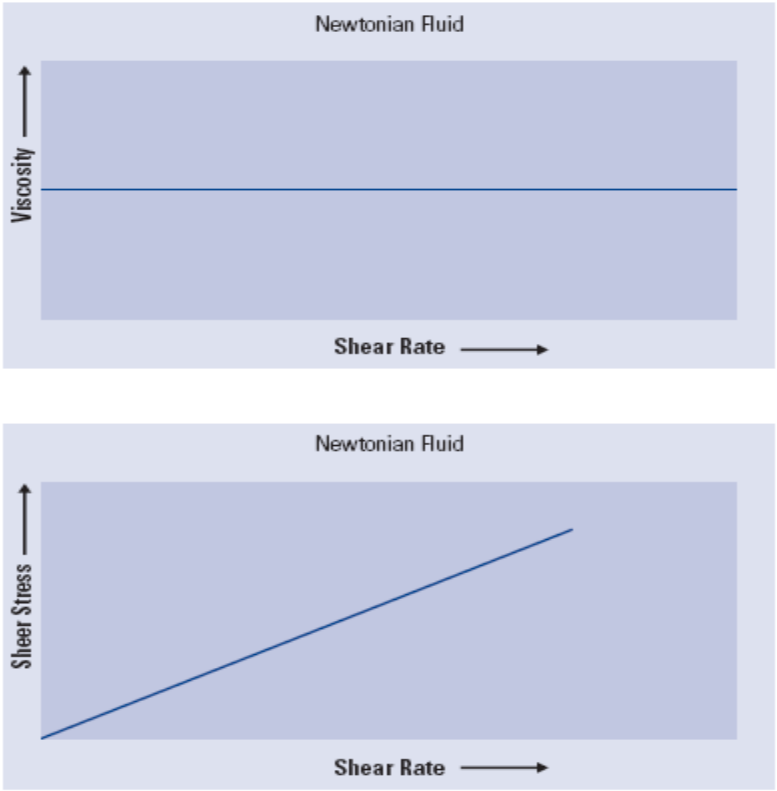


Figure 1.14: Diagram for viscosity of a simple Newtonian fluid

1.4.10.2.2 Non-Newtonian flow

1.4.10.2.2.1 Plastic Flow

Most materials do not exhibit Newtonian flow after the yield, but have a viscosity that decreases (shear thinning) until a plateau is reached Fig (1.15). Lipsticks, drilling muds and toothpaste are good examples of shear thinning non-Newtonian materials with a yield stress.

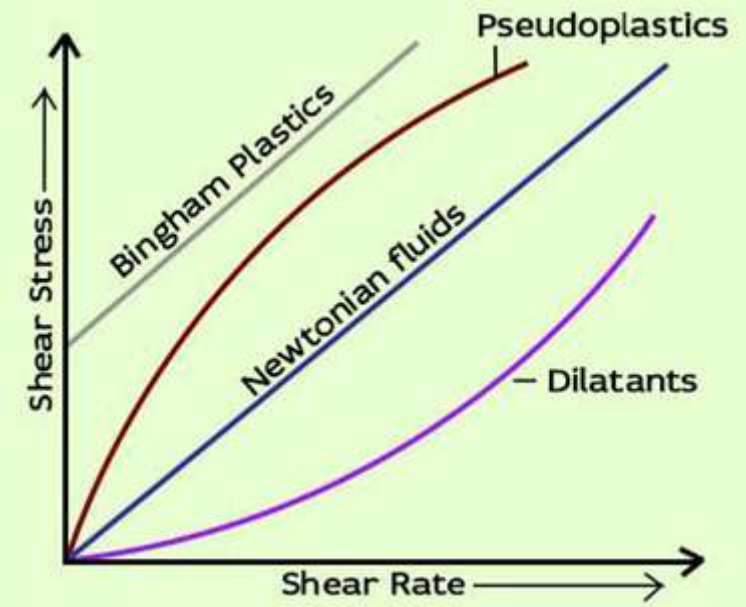


Fig 1.15: curves for typical time-independent fluids.

1.4.10.2.2.2 Pseudoplasticity

Some materials do not have a yield stress, nevertheless they behave nonlinear. These are considered pseudoplastic. They flow instantaneously upon application of stress but also display shear thinning behavior Fig (1.16). Polymer solutions exhibit pseudoplastic flow as does bread dough and many paints and cosmetics.

1.4.10.2.2.3 Dilatancy

Dilatancy, also known as shear thickening Fig (1.16), is an unusual phenomenon whereby materials actually increase their viscosity upon stirring or shearing. In some cases these are dense suspensions of solid particles in a fluid medium, which develop greater spacing between particles during agitation. This behavior is infamous in quicksand, moist beach sand

and certain pharmaceuticals such as a suspension of penicillin. Shear thickening often result from material instability and structure rearrangements or phase separation.

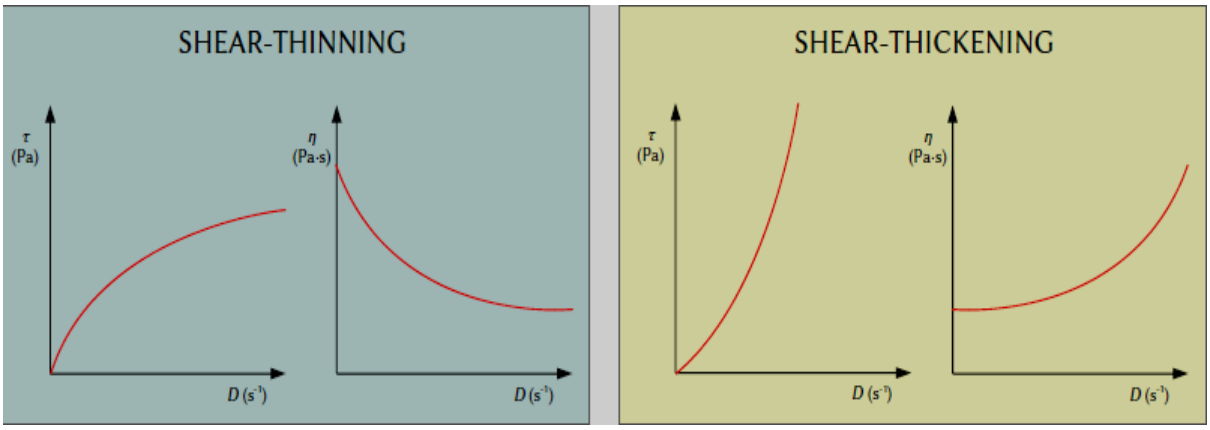


Fig 1.16: Diagram for shear thinning and shear thickening.

1.4.10.2.2.4 Thixotropy

Viscosity decreases over time under constant shear rate. As shear rate decreases the material will gradually recover the original internal structure before shear, this can take seconds or days for full recover. Many times, when a material sits, it will "structure", this will give a "false" high viscosity reading if the material is not premixed.

1.4.10.2.2.5 Rheopexy

Whereas a thixotropic fluid's viscosity decreases over time under constant shear rate, a rheopectic fluid's viscosity increases under shear. A rheopectic fluid such as a dense suspension of latex particles or plastisols will gel when agitated. If allowed to rest, a rheopectic fluid will return to its original lower viscosity.

1.4.10.3 Dynamic oscillatory testing

1.4.10.3.1 Linear viscoelastic measurement

Oscillatory deformation testing is used to investigate the viscoelasticity of a sample and probes its structure before it begins to flow. Most inks, paints and coatings are not completely solid or completely liquid but instead exhibit viscoelastic properties. Under certain conditions they flow, essential for application and in other circumstances they do not, helping product control.

In oscillation experiments the rheometer is used to determine whether the material has greater viscous tendencies (more likely to flow) or is predominantly elastic with more structural stability and also which behavior will dominate under specific conditions. Viscoelasticity is a time-dependent property in which a material under stress produces both a viscous and an elastic response **(**Goodwin *et al*., 2008).

The G' value is a measure of the deformation energy stored in the sample during the shear process. After the load is removed, this energy is completely available and acts as a driving force for the reformation which partially or completely compensates the previous deformation. Energy storing materials display reversible deformation behaviour if they remained in an unchanged form after the load cycle. Thus, G' represent the elastic behavior of a sample.

The G'' value is a measure of the deformation energy used up in the sample during the shear process. This energy is either used up during the process of changing the sample's structure or dissipated into the surrounding environment in the form of heat. Energy losing materials display irreversible deformation behaviour if they occur in a changed form after the load cycle. Thus, G'' represent the viscous behaviour of the sample.

The loss factor (tan δ) is calculated as the lost and the stored deformation energy. It therefore reveals the ratio of the viscous to the elastic portion of the deformation behaviour. Ideal elastic behaviour is expressed as $\delta = 0^o$, or $\tan \delta = 0$, because here G' completely dominates G'', while ideal viscous behavior is expressed as $\delta = 90^o$, or $\tan \delta = \infty$, because here G'' completely dominates G'. When viscous and elastic behavior exactly balance (i.e. when $G' = G''$), then $\tan \delta = 1$ or $\delta = 45^o$, and a crossover of G' and G'' values was noticed at a certain frequency.

The crossover frequency provides a good indication of the viscoelastic behaviour of the material. Materials with a lower crossover value generally implied a higher elastic contribution to their viscoelastic properties (Ramachandran, *et al.*, 1999). A typical gel behaviour is indicated by a higher G' than G'' throughout the frequency range tested.

For samples that display shear-thinning behavior, the shear viscosity is dependent on the degree of shear load. The flow curve shows a decreasing curve slope, i.e. the viscosity decrease with increasing load. Most polymers are pseudoplastic and thixotropic. E.g. of shear-thinning samples: some polymer solutions, polymer melts, paints, glues, shampoos. The structure in polymer with shear-thinning behaviour is most probably a three dimensional coil. The shape of each coil appears to be roughly spherical and each one is entangled many times with neighbouring macromolecules. During the shearing process, the molecules are

oriented into the shear direction as well as the shear gradient direction. In doing this the molecules disentangle to a certain extent, which lower their flow resistance and hence increases the freedom of movement of individual chains. In polymer solutions with very low concentrations, the macromolecule chains may even become completely disentangled. The molecules, which are now oriented to a high degree, are then separate and do not touch each other. On the other hand the shear viscosity of a shear-thickening (or dilatants) sample is also dependent on the degree of shear load, but during shearing, the extent of entanglement with neighbouring macromolecules is greater than that of disentanglement and hence flow resistance increases and consequently viscosity increases with increasing load i.e. the flow curve shows an increasing curve slope, e.g. of shear - thickening samples: dispersions with a high concentration of solids or polymers; such as ceramic suspensions, starch dispersions, plastisol pastes (with insufficient softener content) (Mezger, 2002).

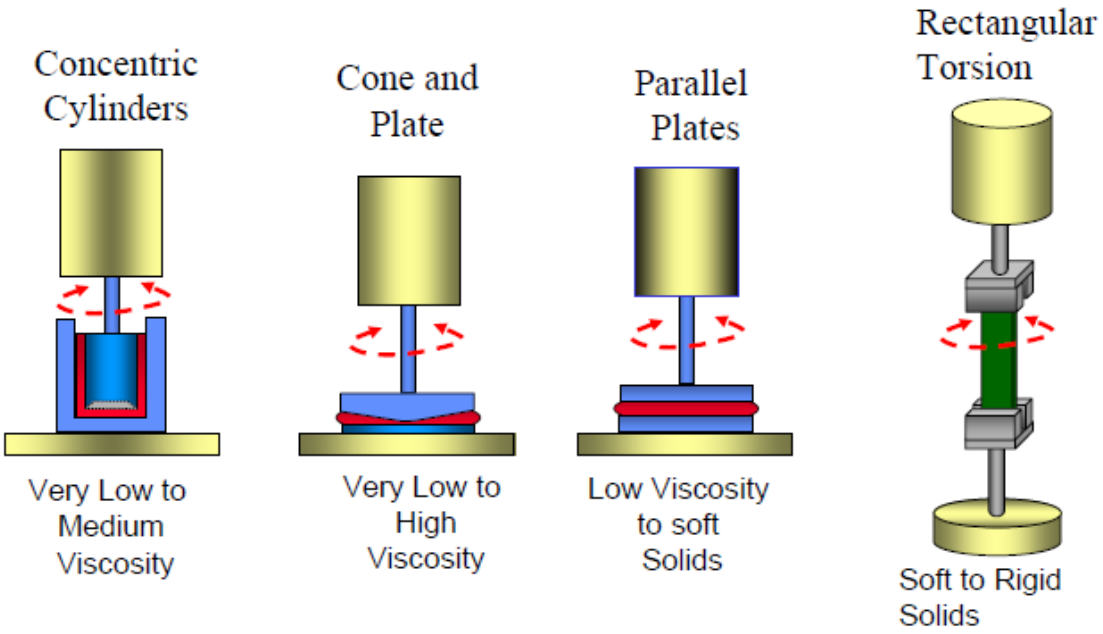


Fig 1.17: Schematic diagram of basic tool geometries for the rotational rheometer.

1.4.10.4 Rheology Applications

Rheology has applications in materials science engineering, geophysics, physiology, human biology and pharmaceutics. The science of rheology and the characterization of viscoelastic properties in the production and use of polymeric materials have been critical for the production of many products for use in both the industrial and military sectors. Study of flow properties of liquids is important for pharmacists working in the manufacture of several dosage forms, such as simple liquids, ointments, creams, pastes etc. The flow behavior of

liquids under applied stress is of great relevance in the field of pharmacy. Flow properties are used as important quality control tools to maintain the superiority of the product and reduce batch to batch variations.

Food rheology is important in the manufacture and processing of food products. Food rheology is the study of the rheological properties of food, that is, the consistency and flow of food under tightly specified conditions. The consistency, degree of fluidity, and other mechanical properties are important in understanding how long food can be stored, how stable it will remain, and in determining food texture. The acceptability of food products to the consumer is often determined by food texture, such as how spreadable and creamy a food product is.

Food rheology is important in quality control during food manufacture and processing. Thickening agents, or thickeners, are substances which, when added to an aqueous mixture, increase its viscosity without substantially modifying its other properties, such as taste. They provide body, increase stability, and improve suspension of added ingredients. Thickening agents are often used as food additives and in cosmetics and personal hygiene products. Some thickening agents are gelling agents, forming a gel. The agents are materials used to thicken and stabilize liquid solutions, emulsions, and suspensions. They dissolve in the liquid phase as a colloid mixture that forms a weakly cohesive internal structure. Food thickeners frequently are based on either polysaccharides (starches, vegetable gums, and pectin), or proteins (Braun *et al*., 2000, Schramm, 2000).

1.4.10.5 Rheological properties of gums

It has been reported that gum arabic solutions show Newtonian flow behaviour in the shear rate range from $50s^{-1}$ to $100s^{-1}$, even at gum arabic concentration as low as 4% (Mothé *et al*., 1999). In the shear rate range from 1 s^{-1} to $50s^{-1}$, the shear thinning behavior has been observed. In the gum arabic concentration range between 10 and 25%, gum arabic solutions show a significant shear thinning behavior, as compared with that of solutions with concentration in the range 30-50 % (Mothé *et al*., 1999). Time dependent thickening flow behavior has been observed for 3 wt% gum arabic solutions at shear rate below 1 s^{-1}. Gum arabic solutions have shown shear thinning behavior at concentrations between 3 and 32 wt% (Sanchez *et al*., 2002). Gum solution above 30% shows higher solution viscosity and exhibits pseudoplasticity (Williams *et al*., 1990).

The rheological complexity of gum arabic is also evident from the increase of viscosity/elasticity with time, which has been attributed to the interfacial activity of gum arabic, gradually developing an elastic interfacial film between samples and measuring geometry (Sanchez *et al*., 2002). The sample solutions investigated by Sanchez *et al*. were centrifuged at 24500 rpm for 30 min to remove air bubbles and insoluble material. The air bubbles contained in gum arabic solutions significantly influence the rheological properties of the solutions (Tanaka *et al*., 2006).

The effects of molecular association on rheological behavior of gum arabic solution were investigated by transient shear stress (Li *et al*., 2011). Thixotropic behavior was observed in gum arabic solution for the first time. The effect of shear history was observed, which arose from the difference of the amount of molecular associations in gum arabic solution. The molecular associations were resumable with given sufficient rest time. Stress jump experiments were able to distinguish the effects of molecular association on the hydrodynamic (viscous) and structural (elastic) contributions to the total stress. At low shear rates, the stress is dominated by the elastic contribution and the apparent stress is elastic-like, reflecting the existence of a larger number of molecular associations in the solution. When the shear rate was higher than $10s^{-1}$, only the viscous contribution is dominant with complete breakdown of molecular association. At this condition gum arabic solution shows a purely viscous and Newtonian behavior. The elastic and viscous contributions were also investigated by transient flow during the time evolution. The two contributions increased with rest time, but the elastic contribution increased faster than the viscous contribution, indicating a fast rate of buildup of molecular associations of gum arabic. Thus the presence of molecular associations in gum arabic solutions induced the deviation from a Newtonian behavior, so showing thixotropy for gum arabic solution at low shear rates. The elastic contribution to stress was strongly influenced by the existence of molecular association (Li *et al*., 2011).

The interfacial rheology of diluted solutions of gum arabic at liquid air interface was first studied by Warburton (1966) and subsequently received further attention due to the relationship of this property to emulsification performance (Dickinson *et al*., 1988, Fauconnier *et al*., 2000). From his results Warburton (1966) concluded that the gum arabic molecule is spheroidal or flat cylinder-like in shape, rather than a long chain. Sharrif and Warburton (1974), using an oscillatory surface rheometer, found that the interfacial elasticity of *A. senegal* increased with increasing concentration and ageing time.

Moules *et al*., (1990) using an oscillating ringsurface rheometer investigated the interfacial rheology of aqueous solutions of differing concentrations of *A. senegal* gum over a period of

5 min. They found that the interfacial elasticity increased as a function of both concentration and time and predicted that the growth of film would continue over many hours and may indeed never stop completely. Burgess *et al*., (1997) observed a strong elastic film for gum arabic solutions using a Mark II surface rheometer and noted a reduction in the surface elasticity and an increase in the surface tension when it was mixed with bovine serum albumin (BSA). This was attributed to a possible interaction between the glucuronic acid groups in gum arabic and the BSA which resulted in the formation of insoluble complex.

Dickinson *et al*., (1989) have suggested a strong correlation between the amount of proteinaceous components within gum arabic and the surface rheology. Elmanan *et al*., (2008) have concluded that both *A. senegal* and *A. seyal* have surface active properties. The higher interfacial elasticity and viscosity generated by *A. senegal* compared to *A. seyal* would appear to be associated with the difference in nature and distribution of the protein between the two gums. The total amount of protein is less for *A. seyal* than for *A. senegal* and the effective high molecular weight protein, part of which is arabinogalactan protein (AGP) is not so accessible in *A. seyal*. This difference could account for their surface behaviour and for the greater effect shown by *A. senegal*.

1.5 Objectives

The objectives of this study are:-

1. To investigate the physicochemical characteristics of the gum exudates from *Acacia nilotica* var. *adstringens.*

2. To study the molecular weight and molecular weight distribution of *A. nilotica* var. *adstringens* gum.

3. To compare the physicochemical properties of *A. nilotica* var. *adstringens, A. nilotica* var. *nilotica,* and *A. nilotica* var. *tomentosa* gum.

4. To study the emulsification properties of *Acacia nilotica* var. *adstringens* gum.

5. To study the rheological properties of *Acacia nilotica* var. *adstringens* gum.

CHAPTER TWO

Materials and Methods

2.1Materials

2.1.1 Gum samples

In total 45 samples of authentic *Acacia nilotica* var. *adstringens* gum Fig (2.1) were collected from fifteen different trees from Elain forest which lies between latitude 12^{o} $52^{/}$ and 13^{o} $03^{/}$ north and is situated about 26 Kms South east of Elobied town North Kordofan state , Sudan during season 2016-2017.

The samples were classified into groups:

- Three samples were made from each tree.
- One composite sample from each three samples was made (the number of samples were fifteen samples).
- One composite sample was made from each three composite samples (five samples from fifteen composite samples).
- One whole composite sample was mad from three composite samples.
- Composite samples were prepared by mixing equal weights from each sample, taken from each tree, considering the moisture content of gum samples.

Details of the gum samples are given in Table (2.1)

Table 2.1: Sample code, location, date of collection, soil type and rain fall of *Acacia nilotica* var. *adstringens* gum season 2016.

Code	Location		Date of collection	Type of soil	Rain fall
	State	Specific area			
T1	NthorKordofan	Elain forest	Jan-016	Clay	150-450mm
T2	NthorKordofan	Elain forest	Jan-016	Clay	150-450mm
T3	NthorKordofan	Elain forest	Jan-016	Clay	150-450mm
Comp C1	is prepared by mixing equal amounts of (T1+T2+T3)				
T4	NthorKordofan	Elain forest	Feb-016	Clay	150-450mm
T5	NthorKordofan	Elain forest	Feb-016	Clay	150-450mm
T6	NthorKordofan	Elain forest	Feb-016	Clay	150-450mm
Comp C2	is prepared by mixing equal amounts of (T4+T5+T6)				
T7	NthorKordofan	Elain forest	Mar-016	Clay	150-450mm
T8	NthorKordofan	Elain forest	Mar-016	Clay	150-450mm
T9	NthorKordofan	Elain forest	Mar-016	Clay	150-450mm
CompC3	is prepared by mixing equal amounts of (T7+T8+T9)				
T10	NthorKordofan	Elain forest	Mar-016	Clay	150-450mm
T11	NthorKordofan	Elain forest	Mar-016	Clay	150-450mm
T12	NthorKordofan	Elain forest	Mar-016	Clay	150-450mm
Comp C4	is prepared by mixing equal amounts of (T10+T11+T12)				
T13	NthorKordofan	Elain forest	Aprr-016	Clay	150-450mm
T14	NthorKordofan	Elain forest	Apr-016	Clay	150-450mm
T15	NthorKordofan	Elain forest	Apr-016	Clay	150-450mm
Comp C5	is prepared by mixing equal amounts of (T13+T14+T15)				
Comp CW	is prepared by mixing equal amounts of (Comp C1 + CompC2 + Comp C3 + Comp C4 + Comp C5)				

Fig 2.1: *Acacia nilotica* var.*adstringens* gum.

2.1.2 Purification of crude gum

The gum samples used in this study were dried under shade and cleaned to remove impurities such as wood pieces and sand particles were carefully removed, then rinse with distilled water and redried. Then each sample was reduced to a fine powder using a mortar and pestle and sieved using sieve No. 16 then kept in labeled self sealing polyethylene bags.

2.2 Methods

2.2.1 Determination of moisture content

Moisture content of the gum samples was determined according to AOAC, (1980) method. 0.5 gram of gum sample was accurately weighted in a clean, dry preweighted shallow weighing dish. The weighted dish and its contents were dried in an oven (Memmert oven-Beschic Loading Modell 100-800) at 105^{o}C for five hours, then cooled in a desiccator and reweighed. The loss on drying was calculated as follows:

Moisture content (%) = $\frac{W_1 - W_2}{W_1} \times 100$

W_1 = Original weight of sample (g).

W_2 = Weight of sample after drying (g).

2.2.2 pH measurement

pH meter (HANNA-Germany) was calibrated using two different buffers one adjusted at pH 4 and other at pH 11. It was used for determination the pH of the gum fractions, of 1g/100 ml aqueous solution (w/v on dry weight basis).

2.2.3 Determination of ash content

3.0 grams of the dried sample were accurately weighted on dry porcelain crucible and ignited at 550^0C in a muffle furnace (CARBOLITE- Inspection – Repair – Colibration CWF 1100) until free from carbon, cooled in a desiccators and reweighed. Then the total ash % was calculated as follows: (FAO, 1991).

Ash (%) = $\frac{W_3 - W_1}{W_2 - W_1} \times 100$

Where:

W_1 = Weight of the empty crucible (g).

W_2 = Weight of the crucible + the sample (g).

W_3 = Weight of the crucible + ash (g).

2.2.4 Determination of intrinsic viscosity

Viscosity measurement was carried out using Ostwald viscometer contained in water path at 25 ± 1^oC. Gum samples were dissolved in 0.2M; sodium chloride solution to give solution with concentration of 4% .The solution was filtered through 0.8 µm cellulose nitrate membrane filter paper. The viscometer was cleaned by washing with distilled water and dried in acetone. The viscometer was immersed in a water bath adjusted at 25^{o}C ±1 and left to attain equilibrium. The efflux time of solvent and that of the test solution was measured by inserting exactly 15 ml in to the reservoir of the viscometer using glass pipette. Each gum solution was diluted several times and the flow time of the diluted solution was measured. The readings were taken in duplicate and viscosity was calculated according to the equations as described in the previous section.

2.2.5 Determination of specific optical rotation $[\alpha]_D^T$

The optical rotation was determined for 1.0% solution on dry weight basis, the sample was dissolved in distilled water, mixing on a roller mixer until the sample fully dissolved (approximately 5 hours), after filtration of the gum solution through whatman cellulose nitrate membrane filter paper (0.8μm), optical rotation was measured at room temperature (25°C) using polarimeter (Bellingham + Stanley optical) ADP 410 with a D-line of Na (589.3nm) fitted with a cell of path length of 20.0 cm. The specific optical rotation was calculated according to the relationship:

Specific optical rotation $[\alpha]_D^T = \frac{\alpha \, x \, 100}{L \, x \, C}$

Where:

α = Observed angle of rotation.

L = the length of sample holder in decimeters (dm).

C = concentration in gm/100ml.

T = Temperature.

2.2.6 Nitrogen and protein Content

The Kjeldahl method was used to determine total nitrogen in gum samples according to AOAC (1990). The method consists of three basic steps: (1) digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia, (2) distillation of the ammonia into a trapping solution, and (3) quantification of the ammonia by titration with a standard solution. The reactions involved in these steps can be shown as follows:

- Sample + H_2SO_4 (conc.) catalyst +Heat → $(NH_4)_2SO_4$
- $(NH_4)_2SO_4$ + 2 Na OH → $2NH_3 + Na_2SO_4 + 2H_2O$
- $NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$
- $H_2BO_3^- + HCl \rightarrow H_3BO_3 + Cl^-$

2.2.6.1 Method

1 gram of each sample (in duplicate) was weighed and transferred to Kjeldahl digestion tubes (250ml) and Kjeldahl tablet (copper sulphate-potassium sulphate catalyst) was added to each. 13 cm^3 concentrated, nitrogen free, sulphuric acid was added. The rack with tube was inserted under fume hood and installed exhaust manifold connected to water aspirator. Then keep in digestion unit (Gerhardt analytical system, Figure 2.2) at 420^oC until liquid becomes transparent. Rack was removed with exhaust manifold from digester and allowed to cool to room temperature under fume hood, then exhaust manifold was removed and tubes were transferred separately to distillation unit (VAP 200-Gerhardt, Germany) (Figure 2.2). Automatic distillation by addition of about 65ml of distilled water and about 35ml of 40% (w/v) sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 25 cm^3 of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.1M, sulfuric acid using methyl red and bromo cresol green as indicator.

$$\%N = \frac{0.014\ x\ M\ x\ (volume\ of\ titrant - volume\ of\ blank) x\ 100}{weight\ of\ sample\ (grams)}$$

Where:

M is the molarity of sulfuric acid.

Protein content was calculated using nitrogen conversion factor.

% protein = % N x 6.25

Fig 2.2: Kjeldahl units (digestion and distillation units)

2.2.7 Determination of total glucouronic acid

Acid equivalent weight was determined according to the method reported in Encyclopedia of Chemical Technology (1966) with some modification. A cation exchange column was packed with Amberlite IR (120 H^+) resin. 2 molar sulphuric acid was passed through the column until the resin was thoroughly washed with the acid. Then this was followed by distilled water until the column was free from sulphate. 50 ml of 3% w/v gum solution was allowed to pass through the column under gravity action, followed by distilled water until a volume of 250 ml of the eluent and washing were collected and titrated against 0.1N NaOH. The apparent equivalent weight of the acid was calculated as follows:

$$Acid\ equivalent\ weight\ = \frac{weight\ of\ sample\ x\ 1000}{volume\ of\ titrant\ x\ normality\ of\ alkali}$$

$$\%\ Uronic\ acid\ anhydride\ = \frac{194\ x\ 100}{Acid\ equivalent\ weight}$$

Where:

194 Molecular weight of uronic acid.

2.2.8 Determination of cationic composition

2.2.8.1 Method

2.2.8.1.1 Sample preparation

0.5 g of each gum sample was weighted, mixed with 4ml of 70% HNO_3 (Sigma-Aldrich, USA) and 2ml of H_2O_2 (Sigma-Aldrich)), The mixture was digested for 40 min using a Multiwave ECO microwave digestion system Brima (2016) . The samples were transferred to 25 ml volumetric flasks, made to volume using de-ionized water. The elements were determined using ICP-MS instrument.

2.2.8.1.2 Elemental Analysis

An iCAP Q inductively coupled plasma mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) Fig (2.3) was used to determine the cations concentrations in the digested sample solutions. The iCAPQ operating conditions were: RF power 1550 W; cool gas flow rate 14.1 L/min; nebuliser gas flow rate 0.94 L/min; auxiliary gas flow rate 0.79 L/min; dwell time 0.01 s; total time for each measurement 3 min. A stock solution containing the elements of interest was prepared (40, 20, 10, 5 and 1 µg/L) mixed standard (ULTRA Scientific, North Kingstown, RI, USA). An internal standard solution containing Sc was

prepared from a 100 μg/L solution (ULTRA Scientific). The stock solutions were prepared in 1.0% HNO_3.

The analysis was performed following (Brima, 2016). An internal standard solution (scandium, 100μg/L) was introduced online. A quality control mixed standard of a 20 μg/L containing the elements of interest was analysed. The data were processed using Qtegra software (Thermo Fisher, Waltham, MA, USA).

Fig 2.3: **Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) instrument**

Fig 2.4: Microwave Digestion System (Anton Paar- Multiwave ECO)

2.2.9 Determination of sugar composition

HPLC is widely considered to be a technique mainly for biotechnological, biomedical, biochemical research, and for the pharmaceutical industry, is as well widely used in a lot of fields such as cosmetics, energy, environmental, and food industries (Marcrae, 1985).

2.2.9.1 Sample preparation

The samples were hydrolyzed to liberate the sugar residues. Sample was weighed out (0.5g, taking into account the moisture content) and added to 50 cm^3 of 4% H_2SO_4 and incubated at 100 ^0C for 6 hours. Following this, 2g of $BaCO_3$ was added to the solution and left overnight (minimum of 12 hours) to neutralize the solution. After $BaCO_3$ treatment, universal indicator strips were used to ensure that the sample was neutral before proceeding to the next stage. The solution was then centrifuged at 2500 rpm for 10 minutes to allow the Barium Sulphate (formed from neutralizing the H_2SO_4) to settle. The supernatant was removed and filtered through a 0.45 μm Whatman nylon filter and then diluted 1:1 with 80/20 Acetonitrile/buffer. This constituted the final solution of which 1ml was put in a vial (filtered via 0.45 μm filter) prior to injection into HPLC column.

2.2.9.2 Method

The purpose of analyzing the gum samples by HPLC was to determine the relative concentration of each sugar residue present in the sample, namely rhamnose (Rha), arabinose (Ara), galactose (Gal) and glucuronic acid (GlcA).

Before analysis of the gum samples, calibration curves of these sugars were prepared. Stock concentrations of 5 mg cm^{-3} for each sugar were made up by hydrating in 80/20 acetonitrile/buffer for 2 hours. Dilutions of the stock solution achieved four different concentrations for each sugar over a range of 5–1.25 mg cm^{-3}. This allowed four levels for the calibration curve and an average of 3 replicates for each level was used to ensure accuracy. This calibration allowed the determination of the unknown sugar content for the gum samples. The concentration of each sugar was calculated by peak height and expressed as a % of the total sugar content.

2.2.10 Tannin content

The tannin content was determined according to modification of Prussian blue assay originally devised by Price and Butler and subsequently, modified (Graham, 1992). Tannin

content represents “total phenols” or more accurately the “Gallic acid equivalents” as Gallic acid 99% in purity was purchased from sigma Aldrich and used as analytical standard for determining the hydrolysable tannins. Standard solutions was prepared (500 µg/g Gallic acid in distilled water, and diluted to 400, 300, 200, 100, and 50 µg/g). 0.1ml of each solution was dispensed in a 30ml universal. 3ml of distilled water was added vortex mixed for 30sec. 1.0 ml of 0.016M Potassium hexacyano ferrate(III) [$K_3[Fe(CN)_6]$, was added fallowed by 1.0 ml of 0.02M Ferric Chloride ($FeCl_3$), and immediately mixed by vortex mixer 30sec. Exactly 15 min after adding the reagent to the sample, 5 ml of stabilizer(10 ml of 85% phosphoric acid, (H_3PO_3), 1.0 ml of 1% gum Arabic, and 30 ml of distilled water) was added and vortex mixed 30sec, then after15 min The absorbance was read at 700 nm in triplicate for standard solutions, using (Perkin Elmer Lambda XLS+, UV/Vis spectrophotometer). The gum sample was also prepared by adding all reagents. The absorbance was read at 700 nm in triplicate using Perkin Elmer Lambda 40 UV/Vis spectroscopy.

2.2.11 Calorific value

The calorimeter IKA® C1 is used to determination the calorific value of solid and liquid materials according to national and international standards (eg DIN 51900, BS 1016 T5, ISO1928, ASTM 5468, ASTM 5865 and ASTM 4809).The IKA® C1 calorimeter system was calibrated by standard Benzoic acid tabs about 1g (2 Tabs), with cross cal.val. 26461J/g, RSD 0.03%, and LOT SZBD2180V.The temperature was 19° C, the gas pressure (Oxygen) was 30 bar, and the Pump flow of 2700 rpm. Then 0.5g (taking into account the moisture content) of gum sample were weighted and placed into a plastic bag, big bag or small bag which have cross cal. Val. 46383, and 46463 respectively, the bag was covered by rolling it and placed into a decomposition vessel which is surrounded by a water jacket. Then the sample was combusted in an oxygen atmosphere, and the calorific value of the sample was calculated from the resulting increase in the temperature. The final results (Net cal.val.), was calculated by added calorific value of moisture content (%) multiply by cross cal.val. to the cross cal.val.

2.2.12 Fractionation and molecular weight distribution of *Acacia nilotica* var. *adstringens* gum

Fractionation of polymers can be achieved either by destructive methods (degradation) or non destructive methods (physical separation). The non destructive methods may also be

classified as preparative or analytical. In preparative fractionation, discrete fractions can be isolated and purified, while in analytical fractionation the interest is just to obtain information about the distribution of molecular masses in a certain polymer without isolating it into discrete fractions.

Gum has been a subject of investigation of numerous methods of fractionations. During their study of the molecular structure of *Acacia senegal*, Anderson *et al*. (1966) fractionated the gum by a destructive method, they used sequential Smith degradation (Goldstein *et al*., 1965). In such study the gum is subjected to periodate oxidation which result in ring opening and formation of dialdehydes at the peripheral sugar constituents of the polysaccharide accompanied with a loss of quantitative amounts of formic acid in case of the oxidized sugar group being a hexose and formaldehyde in case of the sugar being a pentose. The periodate oxidation is followed by borohydride reduction of the dialdhyde to diols which form intermediate acetals. The acetals are then hydrolysed by mild acid. The procedure ends up with disintegration of the polysaccharide molecule into small saccharide fragments which can be identified using either thin layer chromatography (Anderson *et al*., 1966) or gas liquid chromatography (Aspinall, 1963).

The most popular non destructive preparative methods of fractionation are those based on the different solubility of polymers of different molecular mass when treated with the appropriate solvent. Another preparative fractionation method which has also been used is the air fractionation of the gum, which depends on the hydrophobicity of the fraction (Bsheer, 2006).

The analytical fractionation methods are interns include many methods such as ultracentrifugation or turbidiometry but the most popular ones are the column chromatographic methods particularly: Gel permeation chromatography (GPC), hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IC) (Tager, 1978).

2.2.12.1 Introduction to Gel Permeation Chromatography

GPC is also known as size exclusion chromatography (SEC) or gel filtration chromatography is a technique which separates molecules according to their size (or more accurately according to their hydrodynamic diameter or hydrodynamic volume), by passing a solution through a bed of gel grains. Smaller molecules are able to enter the pores of the media and, therefore, take longer time to elute, whereas larger molecules are excluded from the pores and elute faster Figure (2.5).

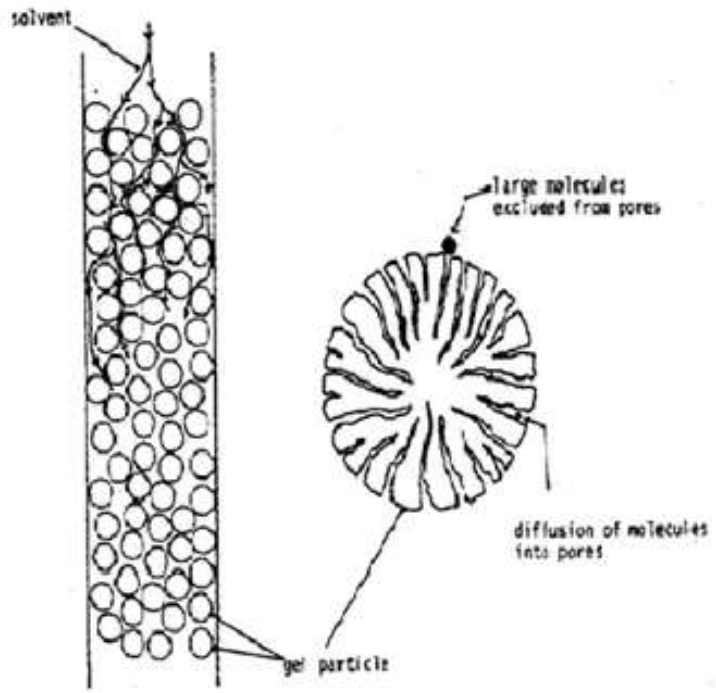


Figure 2.5: Gel permeation chromatography diagram

GPC technique emerged in the 1970s as a premier method for molecular weight distribution (MWD) determination of synthetic and natural polymers. Since that time, GPC as well as traditional types of HPLC (reversed-phase, ion-exchange, hydrophobic-interaction, and affinity chromatography) have been widely used for analysis of proteins and other biopolymers.

Gel permeation chromatography is widely used to determine the molecular mass distribution of macromolecules. GPC, coupled to and on-line, absolute molecular weight determining device (a laser light scattering photometer) and a concentration sensitive detector (such as refractive index) are currently the best available techniques for the quick and absolute determination of polymers molecular weights and their distribution.

The light scattering detector utilizes the principle that the intensity of light scattered elastically by a molecule is directly proportional to the molecular weight (mass detector). By using the refractive index detector (connected directly after the light scattering) it was possible to measure the molecular weight of each fraction as it elutes from the GPC column. In addition, the use of an ultraviolet (UV) detector operated at 214 nm is useful in showing the amount of protein in fractionated material (Figure 2.6).

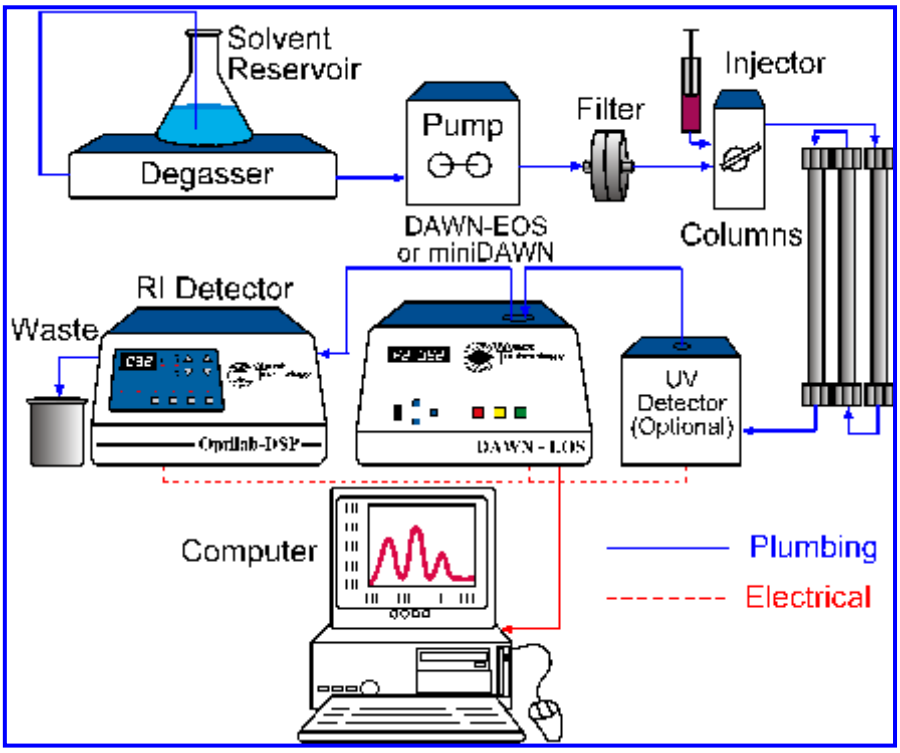


Figure 2.6: Gel permeation column attached to a multi-angle laser light scattering system together with RI, and UV detectors

The typical elution behavior of gum Arabic after GPC fractionated is as follows:

1- The light scattering response shows two distinctive peaks. The first peak has a high response since it corresponds to the high molecular weight material (AGP) content. The second peak is broader with lower response and it accounts for the rest of the gum (90%).

2- The refractive index (RI) response also shows two peaks but the response is opposite to that in light scattering. This it is a Concentration detector and since the AGP is only 10% of the total gum its peaks is smaller that of the AG and GP.

3- The UV response shows three peaks. The first peak is for AGP, which has the protein core, and the carbohydrate attach to it. The second peak appears as a shoulder immediately after the AGP and corresponds to the AG. Finally the third elutes just before the total volume and it corresponds to the GP. The GP peak is not detected on the light scattering (mass detector since it has low molecular weight. Also it cannot be seen on the refractive index (concentration).

AGP could be degraded by proteolytic enzymes, to give molecules with molecular mass similar to the bulk of the gum, and hence, it has been suggested that this fraction has a

Wattle- blossom structure Fig (1.6) where, approximately, five blocks of carbohydrate are attached to a common polypeptide chain (Fenyo *et al*, 1988; Osman *et al.*, 1993). Qi *et al.* (1991) isolated the molecular species of gum Arabic corresponding to AGP by GPC.
fractionation, and following hydrogen fluoride deglycosylation, they concluded that the polypeptide chain consisted of about 400 amino acid residues with a simple empirical formula [Lyp4, Ser 2, Ther, Gly, Leu, His]. This finding was also consistent with Williams *et al.* (2000) findings. Qi (1991) suggested that the molecules were rod like and resembled a twisted hairy rope with small blocks of polysaccharide about 30 residues attached to the peptide chain.

2.2.12.2 Determination of molecular weight parameters, Polydispersity index and radius of gyration using GPC

Using Astra software (Wyatt technology, USA) information about the weight average molecular weight (M_w), molecular weight distribution, and radius of gyration the mass percentage can be obtained.

The molecular weight and radius of gyration are derived using plots such as Zimm (K*C/R(θ)) against $\sin^2(\theta/2)$ and Berry ($\sqrt{K^*C/R\theta}$ against $\sin^2(\theta/2)$. The intercept gives (M_w) and whose slope at low angles gives the root mean square radius ($\langle r_g^2 \rangle^{1/2}$), which is commonly referred to as the radius of gyration.

Multi angle laser light scattering (MALLS) is one of the few absolute methods available for the determination of molecular size over broad ranges. It utilizes the principle that the intensity of light scattered elastically by a molecule (Raleigh scattering is directly proportional to the product of the weight average molecular weight and concentration of the polymer according to the expression:

$$\frac{KC}{R_\theta} = \frac{1}{M_w}[1 + 16\pi^2(r_\theta^2)sin^2(\theta)/3\lambda^2] + 2A_2C \quad \text{..... (1)}$$

The term given between two brackets represent P (θ) which is a general form of a scattering function. K is an optical constant given by

$$K = 4\pi^2 n_0 (dn/dc)^2/\lambda^4 N_A \quad \text{..... (2)}$$

C is the concentration, R_θ is the excess Rayleigh ratio which is the measured quantity, θ the scattering angle, M_w is the weight average molecular weight, A_2 is the second verial coefficient, n_0 is the refractive index of the solvent, dn/dc the refractive index increment of the polymer in solution, λ is the wavelength of light, N_A is the Avogadro's number.

When size exclusion chromatography is coupled to an on-line multi angle laser light scattering (MALLS) detector and a concentration sensitive detector (refractive index or photometric) it is possible to measure the excess Rayleigh ratio, R_θ, of the light scattering intensity and sample concentration (c_i) for each fraction in the fractionated peak. The values of c_i and R_θ are then used to construct a Berry plot of R_θ/Kc against $\sin^2(\theta/2)$ for each slice using first order fit. The value of M_i is then determined from the intercept, of the plot. Thus information about the weight average molecular weight (M_w), number average molecular weight (M_n), molecular weight distribution, polydispersity index (M_w/M_n) and radius of gyration can be obtained, using Eqs. (3 - 5) Astra Software (Wyatt Technology, SB, USA).

Weight average molecular weight

$$M_w = \frac{\sum(c_i M_i)}{\sum c_i} \quad \text{..... (3)}$$

The number average molecular weight

$$M_n = \frac{\sum c_i}{\sum \frac{c_i}{M_i}} \quad \text{..... (4)}$$

The mean square radius of gyration $(r^2)_z$

$$(r^2)_z = \frac{\sum(c_i M_i (r^2)_i)}{\sum(c_i M_i)} \quad \text{..... (5)}$$

The quantities c_i, M_i and $(r^2)_i$ in the above equation are, respectively, the concentration, molecular weight and mean square radius of the i^{th} fraction as described above.

$[R_G]_z^{0.5}$ Can be defined in terms of the distribution of the volume element of the molecule with respect to the square of the distance from its centre of gravity according to the following equation

$$[R_G]_z^{0.5} = \frac{1}{V}\int r^2 dV \quad \text{..... (6)}$$

Although the calculated molecular weight is independent of injected mass, exactly the same concentration was injected for all samples in the current investigation in order to compare the mass recovery for the high molecular weight fraction and the remainder of the gum. The calculation method uses a known dn/dc value and known RI calibration constants. Unlike other methods, no assumption about mass recovery, flow rate and injector accuracy is

necessary in calculating the molecular weight. The refractive index detector (RI) was calibrated using NaCl of known dn/dc (0.174 cm3/g) (Al-Assaf *et al*., 2005).

2.2.12.3 Molecular weight and molecular weight distribution

2.2.12.3.1 Instrumentation

Gel permeation chromatography coupled to a multi-angle laser light scattering detector (GPC-MALLS) system was used to determine the molecular weight and molecular weight distribution. Loading sample injector equipped with 100 ml sample loop.

The system utilises Waters (Division of Millipore, USA) Solvent Delivery System Model 6000A connected to a column containing Superose 6 (Amersharm Biosciences) (10 x 300mm), manual Rheodyne Model 7125 syringe.

The column eluent was monitored by three detectors, refractive index (RI) Wyatt Optilab DSP interferometric refractomter operated at 633 nm (Wyatt Technology Corporation, USA), multi-angle laser light scattering photometer DAWN EOS using He–Ne laser at 690 nm (Wyatt Technology Corporation, USA), and an Agilent 1100 series G1314A UV detector (214 nm, Agilent Technologies) (Al-Assaf *et al*., 2005). RI provides an accurate concentration profile, MALLS enables absolute molecular mass and radius of gyration (Rg), and the UV detects the proteinaceous components of the gum (Katayamaa *et al*., 2006).

The data was processed by the Astra for Windows software (version 4.90.07, Wyatt Technology Corporation).

2.2.12.3.2 Sample preparation

0.02 g in 10ml of 0.2M NaCl (based on dry weight) was prepared, and hydrated by roller (SRT9. Stuart Scientific, UK) mixing the solution overnight to ensure that the sample fully dissolved. The solutions were then centrifuged for 10 minutes at 3000 rpm using Megafuge 1.0R (Heraeus SEPATECH, Germany) centrifuge, filtered using 0.45-µm nylon filter (Whatman, 13 mm) prior to injection into the GPC-MALLS system.

2.2.13 Emulsification properties

2.2.13.1 Materials

- *Acacia nilotica* var. *adstringens* gum 5%.
- Citric acid 0.12% (prepared as a 10% solution in water).
- Sodium benzoate 0.13% (prepared as a 10% solution in water).

- Octanoic/Decanoic acid triglyceride oil, (ODO) 10%.
- Distilled water.

2.2.13.2 Emulsion preparation

Distilled water was added to 8 g of the gum sample (based on dry weight) to becom 40 g in total with a concentration of 20 % (w/w) gum solution. The sample was agitated on a tube roller mixer overnight until the sample completely dissolved. Exact calculated grams for each samples (in the range from about 19.97 to about 20 g) of the prepared gum solution was filtered using 100 µm mesh then mixed with 0.52 ml of 10 % (W/V) sodium benzoate solution as a preservative, and 0.48 ml of 10 % (W/V) citric acid solution to adjust the pH to 4, 15.71ml and 15.73ml distilled water was added, then, 4.2 g ODO oil was added to the gum solution to give a total of 40 g and final concentration of 10%.

The mixed solution was homogenized for 3 minutes using a polytron (PT-2100-KINEMA TICA AC) homogenizer at 22000 rpm. Impeller (PTDA21 9 mm tip diameter) was used as dispersing tool. To achieve small particle size < 1 micron, the pre-emulsified mixture was homogenized using a high-pressure Nanovater (NV30-FA-MITSUBI SHI GOT1000). In order to achieve effective disaggregation of the gum it was passed twice at 75 MPa.

The final emulsion was kept in closed glass universals, then measured emulsion as prepared (amb), then placed at 60^{o}C in the Vacuum Oven (Gallenkamp. OVA031.XX1.5). Droplet size was measured after 3 and 7 days.

2.2.13.2.1 Droplet size analysis

The droplet size distribution of the emulsions was analyzed using Mastersizer 3000, a laser diffraction particle size analyzer (Malvern Instruments). Distilled water was used as dispersant and a value of 1.450 was used for the refractive index for oil phase (ODO). Emulsification stability of samples kept at 60^{o}C was evaluated by particle size change after accelerated stability test for 3 and 7 days. The particle size of the emulsions was described by the volume median diameter (VMD).

Fig 2.7: Mastersizer 3000E Malvern Aimil instrument

2.2.14 Determination of Rheological properties

2.2.14.1 Solutions preparation

The 50% w/w (based on dry weight) gum solutions were prepared in water containing 0.005 % w/v NaN_3 as a preservative. The solutions agitated on a tube roller mixer (SRT9. Stuart Scientific, UK) overnight to ensure that the sample fully dissolves and hydrated. The solutions were then centrifuged for 10 minutes at speed of 3000 rpm using (Megafuge 1.0R, Heraeus SEPATECH, Germany) centrifuge.

A dilute solution (25% w/v) was prepared from stock solution and re-centrifuged as previous procedure and stored at 4^oC prior to investigation of their rheological behavior.

2.2.14.2 Rheological measurements

Rheological measurements were carried out using KINEXUS Pro^+ (Malvern Instruments) fitted with cone and plate geometry (Fig 2.7) with a cone diameter of 40 mm and an angle of 2^0. Steady shear viscosity curves were measured for gum solutions 25 and 50 % w/v both upon shear rate ramp-up (from 0.01 to 10000 s^{-1}) and subsequent shear rate ramp-down (from 10000 back to 0.01 s^{-1}). Dynamic rheological measurements, to determine the elastic modulus (G′), viscous modulus (G″) and dynamic viscosity, were performed in the frequency range of 0.1–10 Hz. The linear viscoelastic region was assessed, at 1 Hz. The temperature of the samples were controlled within 0.1^oC using a Peltier element. The rheometer control and data

processing was done by computer software (Rheology Advantage Data Analysis Program, TA). In all experiments, the samples were covered with a solvent trap to prevent evaporation.

Figure 2.8: KINEXUS Pro+ (Malvern Instruments)

CHAPTER THREE
RESULTS AND DISCUISSION

Table 3.1: Analytical data of *Acacia nilotica* var. *adstringens* gum (all samples)

Sample code	Moisture %	Ash %	pH	Optical rotation $[\alpha]_D^T$ +	Acid equivalent weigh	Gluc uronic acid %
1	**10.78**	**2.13**	**5.11**	**91.5**	**1875**	**10.35**
2	**10.18**	**2.44**	**5.14**	**86.6**	**2000**	**9.70**
3	**10.74**	**2.44**	**5.01**	**104**	**1829.27**	**10.61**
4	**10.76**	**1.17**	**5.00**	**101**	**1948.1**	**9.96**
5	**11.80**	**2.04**	**5.01**	**75.6**	**1875**	**10.35**
6	**10.53**	**2.43**	**5.11**	**98.5**	**1807.23**	**10.73**
7	**10.74**	**1.47**	**5.12**	**101**	**1875**	**10.35**
8	**11.57**	**2.42**	**5.09**	**101**	**1851.85**	**10.48**
9	**10.96**	**1.99**	**5.02**	**99**	**1829.27**	**10.61**
10	**11.77**	**2.04**	**5.10**	**96**	**1829.27**	**10.61**
11	**10.95**	**1.64**	**5.03**	**91**	**1875**	**10.35**
12	**11.95**	**2.43**	**5.05**	**101**	**1807.23**	**10.73**
13	**10.60**	**1.69**	**5.05**	**98**	**2000**	**9.70**
14	**10.70**	**1.99**	**5.01**	**99**	**1829.27**	**10.61**
15	**11.17**	**2.43**	**5.04**	**103**	**1807.23**	**10.73**
Mean	**11.01**	**2.05**	**5.06**	**96.4**	**1869.25**	**10.40**

Table (3.1): Shows six parameters, which are the moisture content, ash content, pH, specific optical rotation, acid equivalent weight and glucuronic acid.

3.1 Moisture content

The moisture content of *A. nilotica* var. *adstringens* ranged between 10.18 and 11.95% with average value of 11.01% Table (3.1). The results show higher moisture content compared to those reported by Karamallah (1999), also it is slightly high to the values reported by Satti

(2011), and agree to the values reported by Alobied (2015) for *A. nilotica* var. *nilotica* gum, and Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum.

3.2 pH value

pH values of *A. nilotica* var. *adstringens* gum. The values ranged between 5.00 and 5.15 with average value of 5.06 Table (3.1). The results were agree with values that obtained by Satti (2011) and Alobied (2015) for *A.nilotica* var. *nilotica*, also agree to results obtained by Siefeldawla (2018) for *A.nilotica* var. *tomentosa* gum, and it is higher than the values obtained by Karamallah (1999) for *A. nilotica* var. *nilotica* and *A. nilotica* var. *adstringens*. It is also higher than that reported for *A. senegal* (Karamalla 1998, 1999 and Ayman 2009) and *A. seyal* by the same author (1999) and Younes (2009), and agree to result obtained by Ayman (2009) for *A. seyal* gum.

3.3 Ash content

The ash content of *A. nilotica* var. *adstringens* which was ranged between 1.17% and 2.4% with average value 2.02% Table (3.1). These results are almost similar to those obtained by Anderson (1977) and Kapoor *et al*., (1991). Also the results are agree with that obtained by Satti (2011) and Alobied (2015) for *A. nilotica* var. *nilotica*, and agree to results obtained by Seifeldawlw (2018) for *A.nilotica* var. *tomentosa* gum .The results are far less than the results reported by Yusuf (2011) for *A. nilotica* var. *nilotica*, he reported an average of 3.54% ash content.

These results are far higher than the results reported by Hassan *et al*., (2005) in the study of *A. seyal* gum, also less than that obtained by Ayman (2009) for *A.senegal* and *A. seyal* gum.

3.4 Intrinsic viscosity

Table (3.1) shows the intrinsic viscosity of *A. nilotica* var. *adstringens* ranged between 10.07 and 11.78 cm^3g^{-1} with the average value 11.05cm^3g^{-1}.

Figs 3.1 to 3.6 show the variation of ηreduced with concentration for composite sample 1 to 5 and all samples composite sample 6.

These results were slightly different to those reported by Satti (2011), and also slightly different from that values obtained by Alobied (2015) and Anderson (1977) for *A. nilotica* var. *nilotica*, also agrees with the results obtained by Siefeldawla (2018) for *A. nilotica* var.

tomentosa gum. These results were far less than the result for Nigerian gum study that obtained by FAO which was 35cm^{3}g^{-1} (Al-Assaf *et al*., 2005).

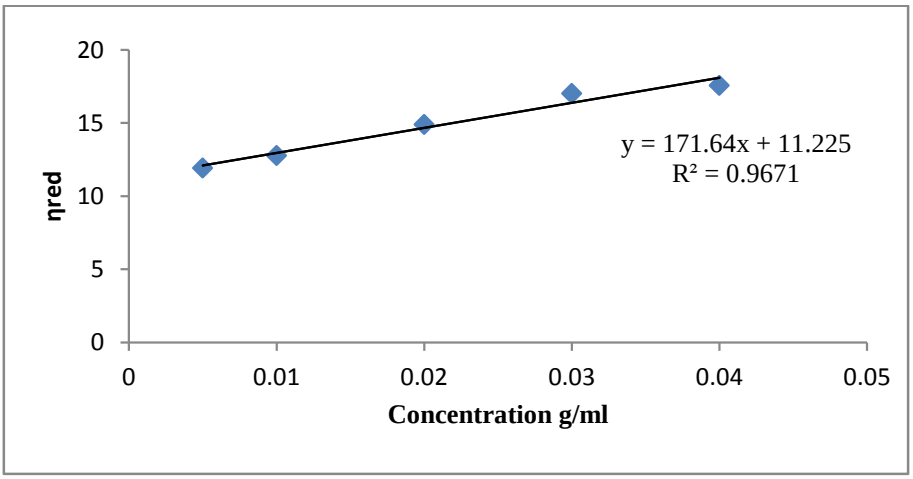


Figure 3.1: Intrinsic viscosity of *A. nilotica* var. *adstringens* gum (Comp C1)

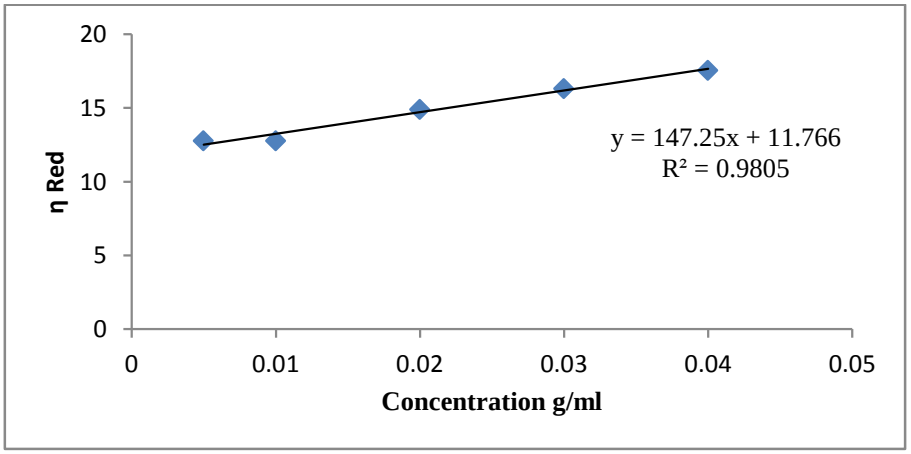


Figure 3.2: Intrinsic viscosity of *A. nilotica* var. *adstringens* gum (Comp C2)

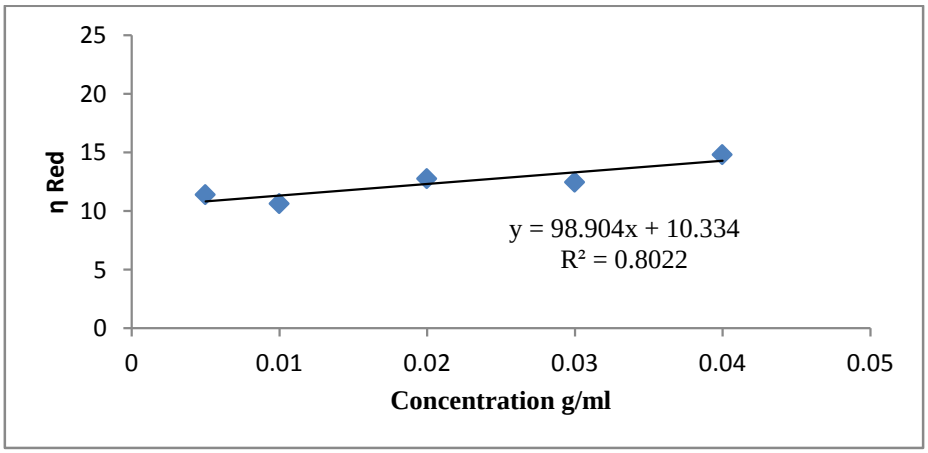


Figure 3.3: Intrinsic viscosity of *A. nilotica* var. *adstringens* gum (Comp C3)

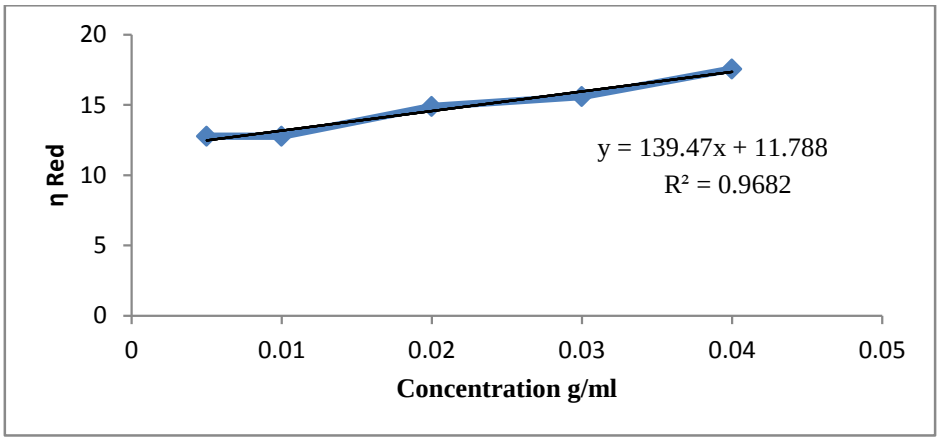


Figure 3.4: Intrinsic viscosity of *A. nilotica* var. *adstringens* gum (Comp C4)

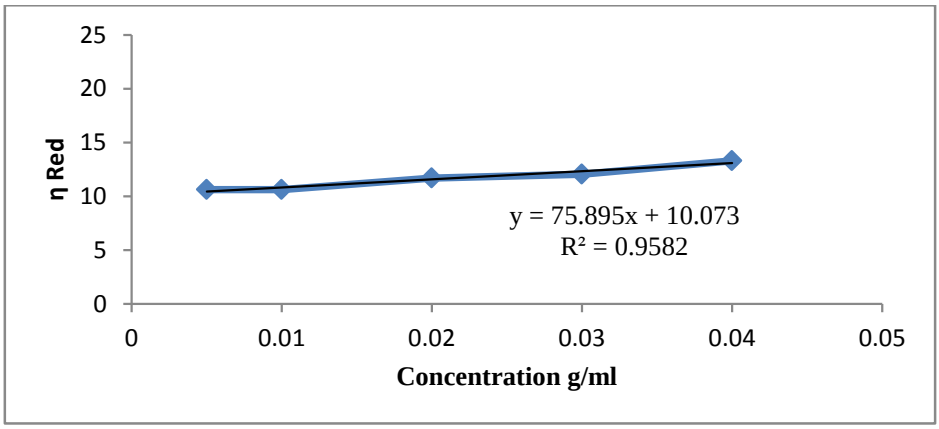


Figure 3.5: Intrinsic viscosity of *A. nilotica* var. *adstringens* gum (Comp C5)

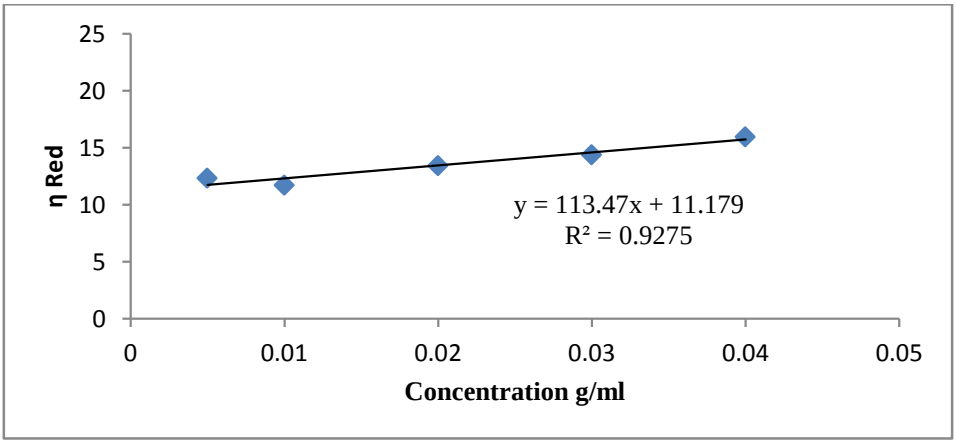


Figure 3.6: Intrinsic viscosity of *A. nilotica* var. *adstringens* gum (Whole comp)

A. nilotica var. *adstringens* gum, have low viscosity compared to *A. senegal* and *A. seyal* gum (Anderson 1983, Jurasek, 1993, Karamallah *et al*., 1998, 2000, Al-Assaf *et al*., 2005, Omer 2006, Ibrahim,2006, Abdelrahman,2008, Elmanan *et al*,.2008 , Younes,2009, Ayman,2009 and Alobied 2015). It is belongs to *Gummeferae* series which distinguished by its low viscosity (Anderson *et al*, 1963, 1966).

3.5 Specific optical rotation

The specific optical rotation is regarded as one of the analytical parameters of which an *Acacia* species gums can be distinguished from other *Acacia* species gums. *Acacia nilotica* is belongs to *Gummeferae* series which distiniguished by positive specific optical rotation.

Specific optical rotation of *A. nilotica* var. *adstringens* gum ranged between + 75.6 and + 104 with average value of + 96.4 Table (3.1).

These results agree well with those reported by Satti (2011) which reported average value + 90.9, + 93.9, and + 99.2 for season 2008, 2009 and 2010 respectively, and Alobied (2015) which he reported average value of+92.6 for *A. nilotica* var. *nilotica* gum. And agree with results obtained by Seifeldawla (2018) for *A.nilotica* var. *tomentosa* gum.

These results agree well with those reported by Karamalla (1999). Interestingly, but they were far more than those obtained by an FAO study for Nigerian gum, (Al-Assaf *et al*., 2005) where a value of +21 was reported. Also higer than that obtained by Karamalla (1999) for *A. seyal*, and higher to that results obtained by Ayman (2009) for *A. seyal*, and different to those obtained by Karamalla (1999) and Ayman (2009) for *A.senegal*.

3.6 Nitrogen and protein content

The percentages of nitrogen and protein content of *A. nilotica* var. *adstringens* gum using kjeldahl method are shown in Table (3.2); the Table shows that the nitrogen percentages of samples ranged between (0.039 % and 0.083 %) with average value 0.06% , and protein percentages ranged between (0.25% and 0.54%) with average value 0.44%. These results are typical to the results obtained by Karamalla (1999) for *A. nilotica* var. *nilotica* and *adstringens* gum, but slightly greater than that obtained by Satti, (2011) and Alobied (2015) for *A. nilotica* var. *nilotica* gum ,and agree with results obtained by Seifeldawla (2018) for *A.nilotica* var. *tomentosa* gum. Also the nitrogen content is greter than that obtained by Anderson (1966, 1977) for *A.nilotica* var. *nilotica* gum. The results are less than that obtained by Karamalla (1999) and Ayman (2009) for *A. senegal* and *A.seyal* gum. The total protein

content was calculated using nitrogen conversion factor (NCF) of 6.51 resulting from amino acid analysis (Satti, 2011).

Table 3.2: Analytical data of *Acacia nilotica* var. *adstringens* gum (composite samples)

Sample code	Moisture %	Ash %	pH	Intrinsic viscosity cm^3g^{-1}	Nitrogen %	Protein %	Acid equivalent weigh	Glucuronic acid %
Comp 1	**11.53**	**2.43**	**5.05**	**11.22**	**0.080**	**0.50**	**1764.71**	**10.99**
Comp 2	**10.60**	**2.42**	**5.00**	**11.76**	**0.054**	**0.36**	**1851.85**	**10.48**
Comp 3	**10.80**	**2.43**	**5.09**	**10.33**	**0.056**	**0.36**	**1829.27**	**10.61**
Comp 4	**11.60**	**2.40**	**5.02**	**11.78**	**0.081**	**0.50**	**1764.71**	**10.99**
Comp 5	**10.58**	**2.01**	**5.12**	**10.07**	**0.083**	**0.51**	**1785.71**	**10.86**
Whole comp	**11.90**	**2.44**	**5.15**	**11.17**	**0.039**	**0.24**	**1807.23**	**10.73**
Average	**11.17**	**2.36**	**5.07**	**11.05**	**0.06**	**0.4**	**1800.58**	**10.78**

3.7 Total glucouronic acid

Acid equivalent weight and total glucouronic acid for *A. nilotica* var. *adstringens* was given in Table (3.1and 3.2). The values of acid equivalent weight are ranged between 1764.71 and 2000, with average value of 1869.25, while uronic acid between 9.70 to 10.99% with average value 10.4%. These values are in agreement with those obtained by (Anderson 1966 and 1977, Satti, 2011, and Alobied, 2015), but the value of uronic acid is far less than that obtained by Kapoor (1991) and Al-Assaf (2005) which are 11.3 and 21% for uronic acid. Also the results are less than that obtained by Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum, and different from the results obtained for *A. senegal* by Osman *et al*., (1993a), Hassan *et al*., (2005), Abdelrahman (2008), Younes (2009) and Ayman (2009), they reported a range of 1040-1660 for acid equivalent weight and 11.89-16.8% for uronic acid, while for *A. seyal* gum they reported a range of 1180-1185.8 for acid equivalent weight and 16.3-16.43% for uronic acid.

3.8 Cationic composition

Cationic composition of *Acacia nilotica* var. *adstringens* gum samples was determined using (ICP-MS); the average values were given in Table (3.3). The results show that calcium, potassium, magnesium and sodium are the four most abundant elements present, and they were in order: Ca > Mg >K > Na. The samples tend to have higher average content of Fe, Mg, Zn, Sr, Mn and Sn. The results also show that elements such as Co, Cr and Pb are present as traces. The order of major elements is similar to the order in *A.senegal* gum obtained by Younes (2009), which have the order: Ca > Mg > K > Na.

Table (3.3) Cationic composition of *Acacia nilotica* var. *adstringens* gum (w/w %)

Sample code	Ca	Mg	K	Na	Mn	Fe	Ni	Cu	Zn	Sr	Sn	Co	Cr	As	Cd	Pb
1	$1.1x10^{-1}$	$5x10^{-2}$	$3.9x10^{-2}$	$4x10^{-2}$	$1.3x10^{-3}$	$2.3x10^{-3}$	$1.4x10^{-4}$	$2x10^{-3}$	$1.9x10^{-3}$	$2.9x10^{-3}$	$2.5x10^{-3}$	$1x10^{-5}$	$8x10^{-5}$	$1x10^{-6}$	$4x10^{-7}$	$1x10^{-5}$
2	$3.1x10^{-1}$	$4x10^{-2}$	$7.8x10^{-2}$	$5x10^{-2}$	$3x10^{-4}$	$2.8x10^{-3}$	$2x10^{-4}$	$9x10^{-4}$	$9x10^{-4}$	$5.6x10^{-4}$	$9.5x10^{-4}$	$1x10^{-5}$	$2x10^{-5}$	$3x10^{-6}$	$3x10^{-6}$	$3x10^{-5}$
3	$3.6x10^{-1}$	$5x10^{-2}$	$4.9x10^{-2}$	$4x10^{-2}$	$4x10^{-3}$	$1.4x10^{-2}$	$1x10^{-4}$	$4x10^{-4}$	$1x10^{-2}$	$4.6x10^{-3}$	$1.8x10^{-3}$	$3x10^{-5}$	$1.1x10^{-6}$	$6x10^{-6}$	$1x10^{-5}$	$1.3x10^{-6}$
4	$3.8x10^{-1}$	$4x10^{-2}$	$3.8x10^{-2}$	$3x10^{-2}$	$2.6x10^{-3}$	$3.5x10^{-3}$	$1.1x10^{-4}$	$3.7x10^{-3}$	$2x10^{-3}$	$4.3x10^{-3}$	$1.3x10^{-2}$	$1x10^{-5}$	$1.1x10^{-6}$	$5x10^{-6}$	$8x10^{-6}$	$5.1x10^{-6}$
5	$3.8x10^{-1}$	$5x10^{-2}$	$2.9x10^{-2}$	$3x10^{-2}$	$4x10^{-3}$	$2.4x10^{-3}$	$1x10^{-4}$	$6x10^{-4}$	$1x10^{-3}$	$5.2x10^{-3}$	$2.1x10^{-3}$	$2x10^{-5}$	$1.0x10^{-6}$	$1.4x10^{-7}$	$1.9x10^{-7}$	$1.6x10^{-6}$
6	$3.4x10^{-1}$	$5x10^{-2}$	$3.8x10^{-2}$	$3x10^{-2}$	$3.8x10^{-3}$	$4.9x10^{-3}$	$9x10^{-5}$	$4x10^{-4}$	$7x10^{-4}$	$4.4x10^{-3}$	$1.6x10^{-3}$	$2x10^{-5}$	$8x10^{-5}$	$6x10^{-6}$	$1.2x10^{-7}$	$1.5x10^{-6}$
7	$3.2x10^{-1}$	$4x10^{-2}$	$3.9x10^{-2}$	$4x10^{-2}$	$3.9x10^{-3}$	$6.5x10^{-3}$	$9x10^{-5}$	$4x10^{-4}$	$7x10^{-4}$	$5.4x10^{-3}$	$1.8x10^{-3}$	$2x10^{-5}$	$1.0x10^{-6}$	$6x10^{-6}$	$1x10^{-5}$	$2.0x10^{-6}$
8	$3.3x10^{-1}$	$5x10^{-2}$	$5.9x10^{-2}$	$4x10^{-2}$	$4.5x10^{-3}$	$3.9x10^{-3}$	$1x10^{-4}$	$5x10^{-4}$	$7x10^{-4}$	$4.9x10^{-3}$	$1.5x10^{-3}$	$2x10^{-5}$	$9x10^{-5}$	$6x10^{-6}$	$1.2x10^{-7}$	$1.8x10^{-6}$
9	$2.3x10^{-1}$	$4x10^{-2}$	$2.8x10^{-2}$	$2x10^{-2}$	$4.5x10^{-3}$	$2.4x10^{-2}$	$1.5x10^{-4}$	$5x10^{-4}$	$6x10^{-4}$	$5.4x10^{-3}$	$2x10^{-3}$	$4x10^{-5}$	$1.8x10^{-6}$	$1x10^{-5}$	$1x10^{-5}$	$2.5x10^{-6}$
10	$7x10^{-2}$	$6x10^{-2}$	$2.9x10^{-2}$	$5x10^{-2}$	$2.3x10^{-3}$	$3.3x10^{-3}$	$1x10^{-4}$	$3.6x10^{-4}$	$6x10^{-4}$	$4.1x10^{-3}$	$1.2x10^{-3}$	$1x10^{-5}$	$9x10^{-5}$	$5x10^{-6}$	$8x10^{-6}$	$9x10^{-5}$
11	$3.9x10^{-1}$	$5x10^{-2}$	$4.8x10^{-2}$	$8x10^{-2}$	$3.2x10^{-3}$	$4.1x10^{-3}$	$6x10^{-5}$	$3x10^{-4}$	$4x10^{-4}$	$3.9x10^{-3}$	$1.5x10^{-3}$	$1x10^{-5}$	$6x10^{-5}$	$4x10^{-6}$	$6x10^{-6}$	$1.4x10^{-6}$
12	$3.3x10^{-1}$	$4x10^{-2}$	$4.5x10^{-2}$	$4x10^{-2}$	$3.3x10^{-3}$	$2.9x10^{-3}$	$4x10^{-5}$	$2.6x10^{-4}$	$4x10^{-4}$	$3.5x10^{-3}$	$2.1x10^{-3}$	$1x10^{-5}$	$5x10^{-5}$	$2x10^{-6}$	$2.2x10^{-7}$	$7x10^{-5}$
13	$3.3x10^{-1}$	$4x10^{-2}$	$5.9x10^{-2}$	$5x10^{-2}$	$1.5x10^{-3}$	$2.5x10^{-3}$	$4x10^{-5}$	$4.3x10^{-4}$	$8x10^{-4}$	$3.9x10^{-3}$	$1.5x10^{-3}$	$3x10^{-5}$	$1.5x10^{-6}$	$6x10^{-6}$	$2.2x10^{-7}$	$8x10^{-5}$
14	$3.7x10^{-1}$	$5x10^{-2}$	$4.7x10^{-2}$	$4x10^{-2}$	$3.2x10^{-3}$	$7.9x10^{-3}$	$1.4x10^{-4}$	$1.9x10^{-4}$	$5x10^{-4}$	$3.5x10^{-3}$	$1.7x10^{-3}$	$1x10^{-5}$	$1.2x10^{-6}$	$3x10^{-6}$	$2x10^{-5}$	$7x10^{-5}$
15	$2.9x10^{-1}$	$5x10^{-2}$	$4.9x10^{-2}$	$5x10^{-2}$	$4.2x10^{-3}$	$2.9x10^{-3}$	$4x10^{-5}$	$2.3x10^{-4}$	$2x10^{-3}$	$3.4x10^{-3}$	$1.7x10^{-3}$	$1x10^{-5}$	$6x10^{-5}$	$3x10^{-6}$	$1.8x10^{-7}$	$7x10^{-5}$
Mean	$3x10^{-1}$	$5x10^{-2}$	$4.5x10^{-2}$	$4x10^{-2}$	$3.1x10^{-3}$	$5.8x10^{-3}$	$9x10^{-5}$	$5.4x10^{-4}$	$2x10^{-3}$	$4x10^{-3}$	$2.4x10^{-3}$	$2x10^{-5}$	$9x10^{-5}$	$5x10^{-6}$	$1.2x10^{-7}$	$1.4x10^{-6}$
Whole comp	$5.3x10^{-1}$	$5x10^{-2}$	$8x10^{-2}$	$7x10^{-2}$	$3.8x10^{-3}$	$4.6x10^{-3}$	$3x10^{-5}$	$1.8x10^{-4}$	$1x10^{-3}$	$4.9x10^{-3}$	$3.4x10^{-3}$	$1x10^{-5}$	$4x10^{-5}$	$1x10^{-5}$	$2x10^{-7}$	$4x10^{-5}$

3.9 Sugars composition

Sugar contents of *A. nilotica* var. *adstringens* gum were measured using HPLC technique, which were found to be 52% arabinose, 24% galactose and traces of rhamnose Table (3. 4). *A.nilotica* var. *adstringens* gum belonds to *gummiferae* series according to Bentham classification (Anderson, 1974) in which arabinose had a higher percentage than glactose, and the lowest percentage of rhamnose. The results are different from that obtained by Satti (2011) and Alobied (2015) for *A.nilotica* var. *nilotica* gum, and different to that obtained by (Osman *et. al* 1993a, Karamalla, 1999 and Abdelrhman 2008) for *A. senegal* and *A.seyal* gum.

Table 3.4: Sugar composition of *Acacia nilotica* var. *adstringens* gum

Sugar	**%**
Arabinose	**52**
Galactose	**24**
Rhamnose	**< 1**

3.10 Tannin content

The mean value of *A.nilotica* var. *adstringens* gum is 422.6ppm. This value is slightly less than that obtained by Satti (2011) 0.08% for *A. nilotica* var.*nilotica* gum, and greter than value obtained by Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum. Also it less than that obtained by Karamalla (1999) 0.11% for *A.senegal* gum.

3.11 The Calorific value

The calorific value of *A. nilotica* var. *adstringens* gum was given in Table (3.5). The mean value of calorific value was found to be 4.064kcal/g. This result is typical to that obtained by Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum. This value is very low, so that the gum is suitable to use in application in dietary food and nutraceutical products.

Table 3.5: The calorific values of *A. nilotica* var. *adstringens* gum

Sample name	**Sample location**	**Sample weight(g)**	**Bag. Cal. Value J/g**	**Gross cal. value**	**Net.cal value J/g**	**Net.cal value Cal/g**	**Cal. value Kcal/g**
A. adstringes gum	Composite	0.5741	46463	15478	17009.8	4063.7	4.064

3.12 Molecular weight and molecular weight distribution of *Acacia nilotica* var. *adstringens* gum

Figures (3.7, 3.8, 3.9, and 3.10) shows the elution profiles of *A. nilotica* var. *adstringens*, *A. nilotica* var *tomentosa, A. nilotica* var. *nilotica* and *A. Senegal* gum respectively using multi-angle laser light scattering (MALLS). The molecular weight distribution of *A. niloitca* var. *adstringens* gum under investigation was measured for whole gum, and fractionated using GPC technique and *A. senegal* was used as control. The elution profile for *A. nilotica* var. *adstringens* monitored using light scattering, refractive index and UV detection has been previously described (Al-Assaf, 2005).

Fig (3.7) shows the light scattering response reflects the mass and concentration and shows two peaks, the first peak has a high narrow response which it corresponds the high molecular weight (AGP) content and it appeared at the elution volume of (~7.5ml), the second peak is broader with lower response. The RI concentration detector response fig (3.7) shows also two peaks, the first peak has a high narrow response coincided with the light scattering response and corresponding to the minor peak which appeared at the elution volumes of (~ 7.6 ml) and represents about (28.54 %) of total mass. The second peak is broader with lower response corresponding to the major peak which appeared at an elution volume of (~10.4 ml) and (~ 71.46%) of total mass. In *A. Senegal* gum the RI response is opposite to that in light scattering as shown in figure (3.10).

The UV response showed that there is protein associated with the high molecular weight materials and the second peak also appears as shoulder and follows the same LS response. The UV response shows three peaks. The first peak (peak 1) is for the (AGP) which has the protein core and the attached carbohydrate. The second peak (peak 2) appears after the (AGP) and corresponds to the arabinoglactan (AG). The third peak (peak 3) which corresponds to glycoprotein (GP).

The UV response shows a third low molecular weight peak which elutes at ~15-18.5 ml before the total volume of the column (~20ml).

The UV response for *A. senegal* gum show three peaks .The first peak is for the (AGP) which has the protein core and the attached carbohydrate. The second peak appears after the (AGP) and corresponds to the arabinoglactan (AG). The third peak corresponds to glycoprotein (GP).

Fig (3.11) give the cumulative molecular weight distribution plot, which show molecular weight fractions content (>10^6). Fig (3.12) shows molar mass and differential root mean of square radius for gum.

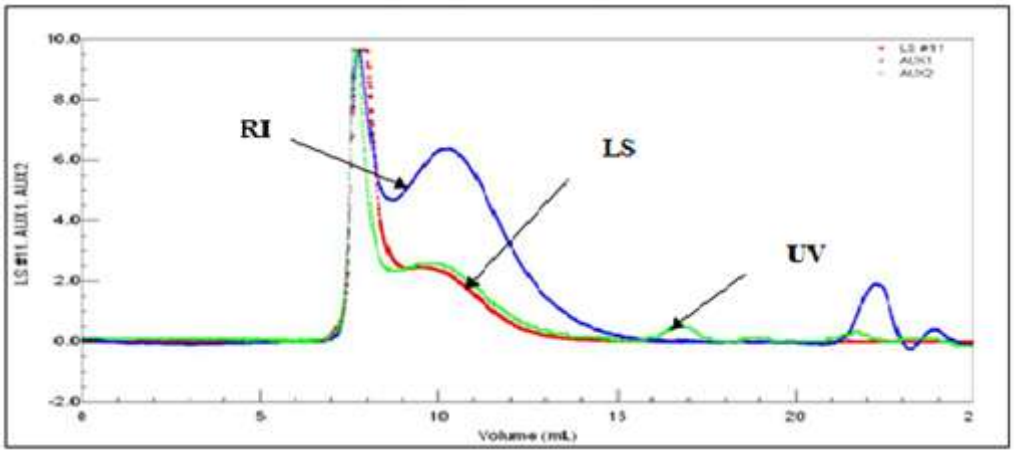


Fig 3.7: GPC chromatogram of *A. nilotica* var. *adstringens* gum.

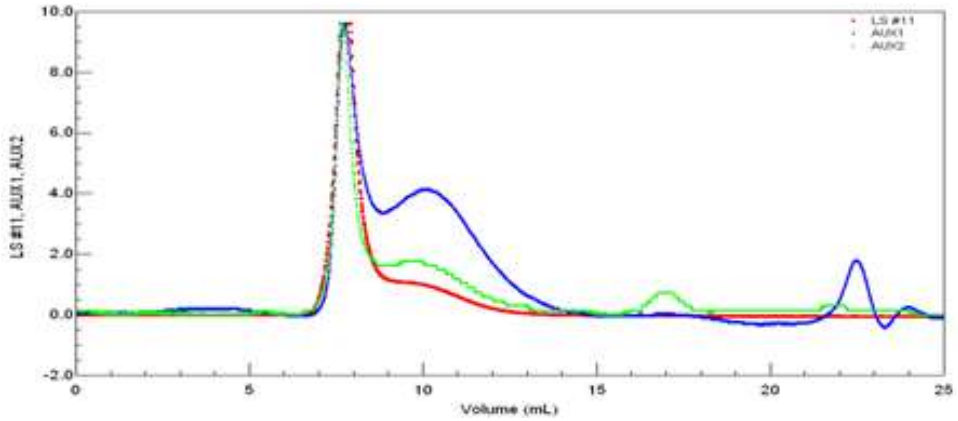


Fig 3.8: GPC chromatogram of *A.nilotica* var. *tomentosa* gum Seifeldawla (2018).

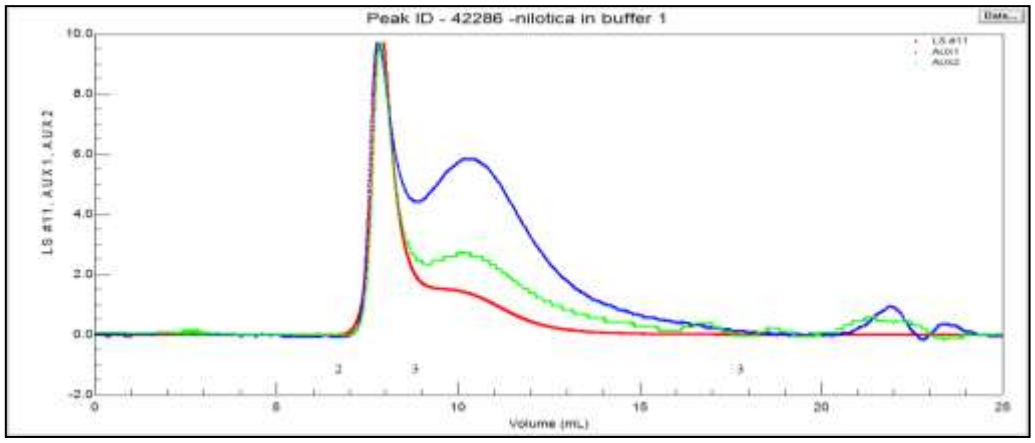


Fig 3.9: GPC chromatogram of *A. nilotica* var. *nilotica* gum, Satti (2011).

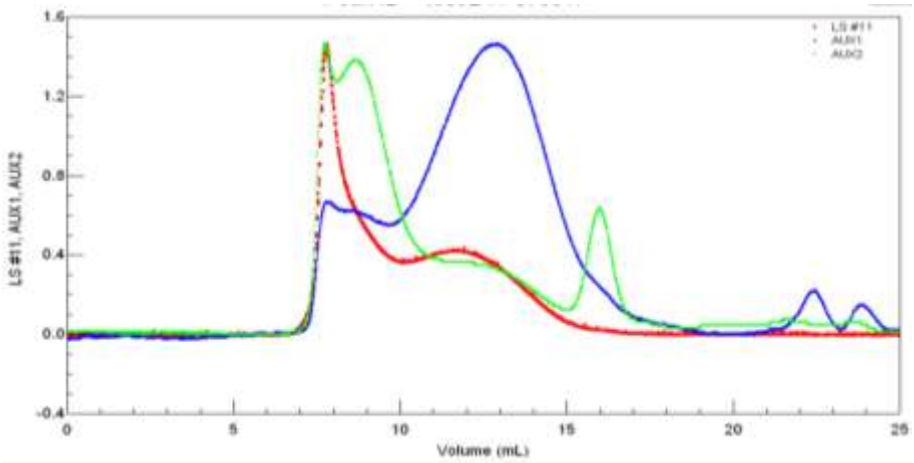


Fig 3.10: GPC chromatogram of *A. Senegal* (control sample).

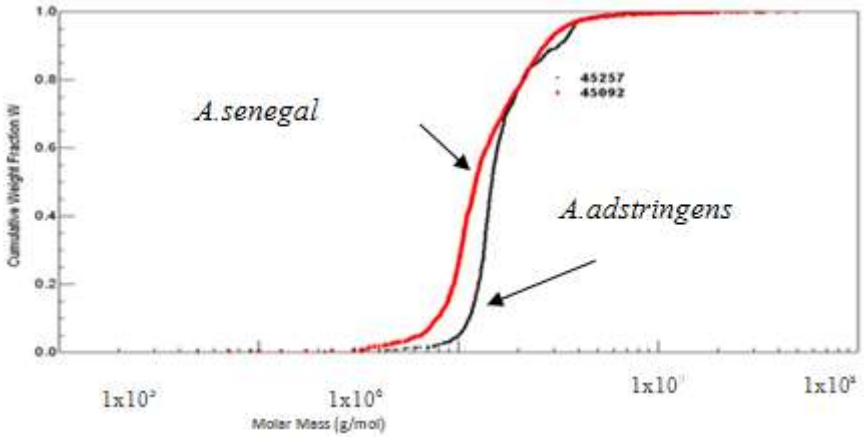


Fig 3.11: cumulative molar mass fraction for *A. nilotica* var. *adstringens* gum (black) and *A. senegal* (red).

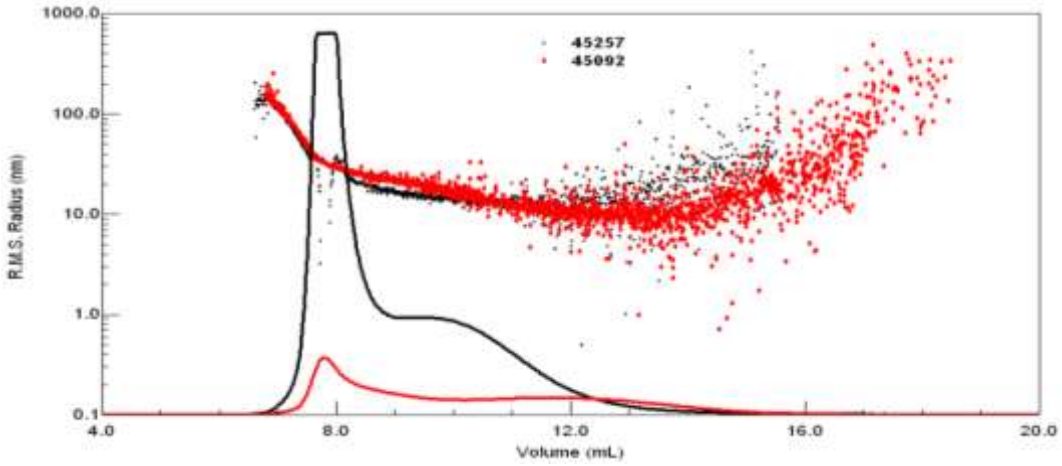


Fig 3.12: differential root mean square radius for *A. nilotica* var. *adstringens* gum (black) and *A.senegal* (red).

The molecular weight was measured for the whole gum as tow peaks; the first peak (which corresponds to AGP) and the second peak (which corresponds to AG and GP) as identified by

refractive index indicator. The molecular weight parameters of *Acacia nilotica* var. *adstringens* gum are given in Table (3.6).

The weight average molecular weight for whole gum was found to be 2.538 $x10^6$ g/mol, with mass recovery of 114.7%. The radius of gyration was 36.6 nm. The AGP component was (28.54%) of total gum with molecular weight of 4.935 $x10^6$g/mol, and radius of gyration 33nm. The (AG and GP) component was found to be 71.46% of the total gum with molecular weight of 1.571 $x10^6$ g/mol. Mass recovery of AGP component is low (28.54%) , this mean a good emulsifying property.

From the results, the weight average molecular weight of whole gum is 2.538$x10^6$ g/mol which is agree to the value reported by Satti (2011) for *Acacia nilotica* var. *nilotica* gum, which ranged from (2.5$x10^6$ to 5.34 $x10^6$) g/mol, and less than value obtained by Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum, he cited value of (2.63 $x10^6$ to 6.7 $x10^6$) g/mol. It is closed to the value that reported by Anderson *et al* (1969) for *A. nilotica* var. *nilotica* gum, they cited a value of 2.27 $x10^6$ g/mol. The molecular weight of *A. nilotica* var. *adstringens* gum obtained from this study is high compared to other gums, such as *A. senegal* and *A. seyal*, Anderson *et al* (1969) reported values of weigh average molecular weights for *A. senegal* is 5.8 $x10^5$ and 6.0 $x10^5$ g/mol, and for *A. seyal* is 8.5 $x10^5$ g/mol. Hassan *et al* (2005) reported value of weight average molecular weight of *A. seyal* is 1.94 $x10^6$ g/mol. Ayman (2009) reported values of weight average molecular weight for *A. senegal* ranged from 1.335 $x10^6$ to 8.078 $x10^5$ g/mol.

The radius of gyration was found to be 36.6 nm, which is agree to the values obtained by Satti (2011) for *A. nilotica* var. *nilotica* gum, which was reported values between 18.8 to 66.9nm. This value is higher than that reported by Al-Assaf *et al* (2005). They cited a value of 26 nm, and higher compared to the value reported for *A. senegal* and *A. seyal* gum, which have an average value of 33.9 nm and 25.7 nm respectively (Hassan *et al*, 2005). Also it is different frome that reported by Siefeldawla (2018) for *A. nilotica* var. *tomentosa* gum which have an average value of 44.3 nm.

The (AGP) molecular weight value was found to be 4.935 $x10^6$ g/mol, this value is slightly lower than the values obtained by Satti (2011) for *A. nilotica* var. *nilotica* gum, which are ranged from 5.83 $x10^6$ to 1.07 $x10^7$ g/mol and Siefeldawla (2018) for *A.nilotica* var. *tomentosa* gum (5.03 $x10^6$ to 8.9 $x10^6$) g/mol. Also it is higher than the value obtained by Al-Assaf *et al* (2005); they cited a value of 1.17 $x10^6$ g/mol. Also it is higher compared to the values of *A. senegal* reported by (Vandevelde and Fenyo, 1985, Randall *et al*, 1989 and Al-

Assaf *et al*, 2005) which were ranged from 1- 4 $x10^6$ /mol. also it is with the range of *A. senegal* gum reported by Osman (2009), he cited values from 3.720 $x10^5$ to 9.905 $x10^6$ g/mol. The amount of AGP in this sample is found to be 28.54%; it is in the range of the values reported by Satti (2011) for *A. nilotica* var. *nilotica* gum, which are ranged from 18.7% to 42.31%, and values obtained by Siefeldawla (2018) which are ranged between 24.5% and 54.7% for *A. nilotica* var. *tomentosa* gum.

The molecular weight of AG component is 1.57 $x10^6$ g/mol. It is agree to the values obtained by Satti (2011) for *A. nilotica* var. *nilotica*, which are ranged from 1.53 $x10^6$ to 2.02 $x10^6$ g/mol, and less than values obtained by Siefeldawla (2018) for *A. nilotica* var. *tomentosa*, which are ranged from 15.1 $x10^6$ to 18.6$x10^6$ g/mol, but it is higher of the value reported by Al- Assaf (2005), which was 3.86 $x10^5$ g/mol. Also it is higher compared to other gums such as *A. senegal* which ranged from 1.5 – 3.0 $x10^5$ g/mol (Al-Assaf *et al*, 2005) and *A. seyal* 1.0$x10^6$ g/mol (Flindt *et al*, 2005, Hassan *et al*, 2005).

Table 3.6: molecular weight parameters of *A. nilotica* var. *adstringens* gum

	Mw/g/mol	**Mass %**	**Rg/ nm**
Total	2.538 $X10^6$	114	36.6
First peak (AGP)	4.935 $X10^6$	28.54	33
Second peak (AG + GP)	15.71 $X10^6$	71.46	-

3.13 Emulsification properties of *Acacia nilotica* var. *adstringens* gum

The particle size distribution for freshly prepared emulsion and after the acceleration test was measured using Mastersizer 3000 (a particle size distribution analyzer).

Table (3.7) show the span% and mean diameters of cumulative droplet distributions (d 0.1, d 0.5 and d 0.9) measurement of *A.nilotica* var. *adstringens* emulsions at different conditions (fresh emulsion and after storage for 3 and 7 days at 60^o C respectively). The fresh emulsion and emulsion stored for 7 days at 60^o C show low degree of polydispersity (span %), indicating good uniformity of the droplet size. The span change varies from 2.537% in fresh emulsion to 2.829% for emulsion stored for 7 days at 60^o C. Fig (3.13) shows the particle size distribution of *A. nilotica* var. *adstringens* emulsion (freshly prepared emulsion, emulsion stored for 3 and 7 days at 60^o C) is constant (~ 1 micron), indicating a stable emulsion. The

particle size of the emulsion was described as volume median diameter (VMD), for *A. nilotica* var. *adstringens* (VMD) was found 0.532µm for fresh emulsion and 0.557µm for emulsion stored for 7 days at 60^{o} C. It is generally known that an increase in droplet mean diameter upon storage is an indicator of droplet coalescence. In relation to emulsion rheology, the occurrence of droplet coalescence will be normally accompanied by a decrease in the emulsion viscosity (Tadros, 2004).

Table 3.7 Span% and mean diameters of cumulative droplet distributions (µm) of freshly and stored (3 and 7 days at 60^{o} C) emulsions of *Acacia nilotica* var. *adstringens* (whole gum)

	Freshly prepared	3 days at 60^{o}C	7 days at 60^{o}C
Span%	2.537	416.2	2.829
d(0.1)	0.0249	0.0487	0.0247
d(0.5)	0.532	1.12	0.557
d(0.9)	1.37	465	1.6

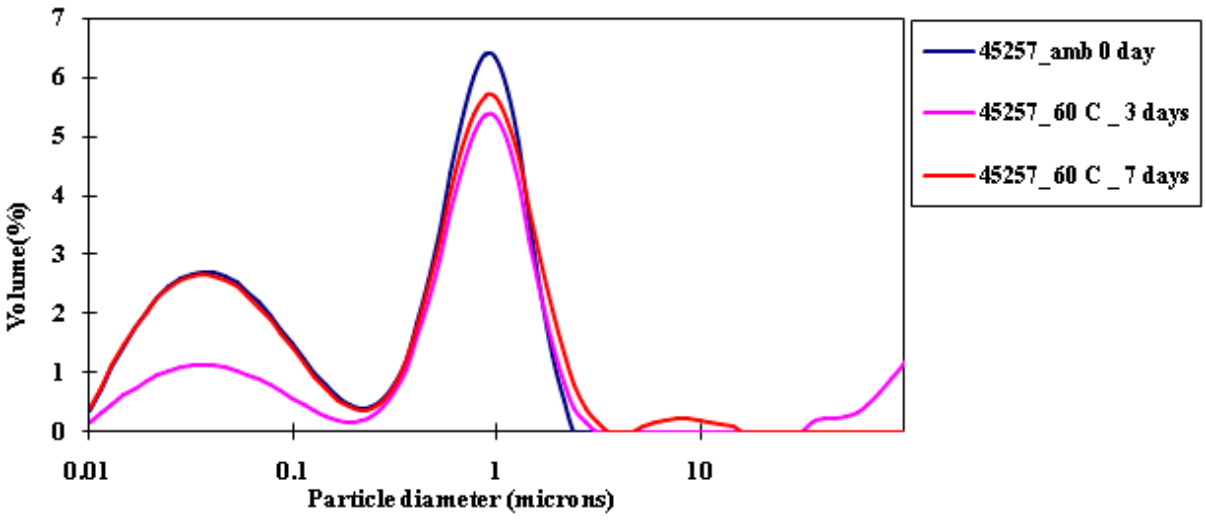


Figure 3.13: Particle size distributions of *Acacia nilotica* var. *adstringens* emulsion samples (Whole gum) at different conditions

3.13.1Emulsion stability index of *Acacia nilotica* var. *adstringens*

Emulsion stability index was taken as a parameter to designate the grade of the gum sample. Therefore, the gum samples which showed a change of 0.7 µm or less were classified as grade 1 (good emulsifier). A change between 0.7–0.85 µm was classified as grade 2. The less stable emulsions which showed a change >0.85 µm were allocated grade 3 (poor emulsifier).

The droplet size distribution before and after accelerated break down procedure is shown in Figure (3.13) and Table (3.8). The Figure shows that gum from *Acacia nilotica* var. *adstringns* has a functional ability to act as emulsifier and stabilizes oil in water emulsion similar to *A.nilotica* var. *nilotica* which studied by Satti (2011) Fig (3.15), *A.nilotica* var. *tomentosa* (Seifeldawla, 2018) Fig (3.16) and *A. senegal.*

Fig (3.14) shows particle size distribution of Acacia nilotica var. adstringens emultion samples (freshly and 7 days at 60 ^{o}C) which clearly showed that no change was found in most emulsions during the incubation for 7 days at 60 ^{o}C.

Table 3.8: Emulsion stability index (ESI, µm) and grade of *Acacia nilotica* var. *adstringens* gum

Initial VMD(µm)	0.532
ATST VMD (µm) after 3days @60^{o}C	1.12
ATST VMD (µm) after 7days @60^{o}C	0.557
ESI (µm)	1.145
Grad	3

* ATST: Accelerated temperature stress test for 3 and7 days at 60^{o} C.

* VMD: Volume Median Diameter.

Acacia nilotica var. *adstringens* gum has a high molecular weight of AGP. The stability of emulsion offered by gum of *A. nilotica* var. *adstringens* may be due to the AGP fraction increase. It established that there is a relationship between the proportion of AGP component

and emulsion stability. It has been suggested that there is some correlation between protein content of gum arabic and interfacial tension at equilibrium (Dickinson *et al*., 1988).

It has been reported (Randall *et al*, 1988 ; Dickinson *et al*, 1989) that the emulsifying activity of gum Arabic at the oil- water interface is attributed to a small amount of protein that is covalently bound to highly branched polysaccharide structure, detected as a high molecular mass. This means that arabinoglactan-protein (AGP) fraction plays a major role in emulsifying in the case of gum Arabic (Islam *et al*, 1997 and Al-Assaf *et al*, 2003). The hydrophilic carbohydrate blocks are linked to the protein chain that strongly adsorbed the oil-water (O/W) interface promoting emulsion stability (William *et al*, 1990).

However, there does not appear to be a direct correlation between nitrogen content and emulsifying effectiveness. Rather it appears that it is the proportion of the three main components which controls the emulsification behaviour (Dickinson *et al*., 1988 and Dickinson *et al*., 1991). Since the protein content of *Acacia nilotica* var. *adstringens* gum is very small (0.4%) compared to *Acacia senegal* gum (~2.4%), the gum has high effective emulsification, and the most suitable explanation of the high stability of emulsion offered by the gum of *Acacia nilotica* var. *adstringens* is a "Thin Oblate Ellipsoid" model of arabinogalactan fraction (Sanchez *et al*., 2008).

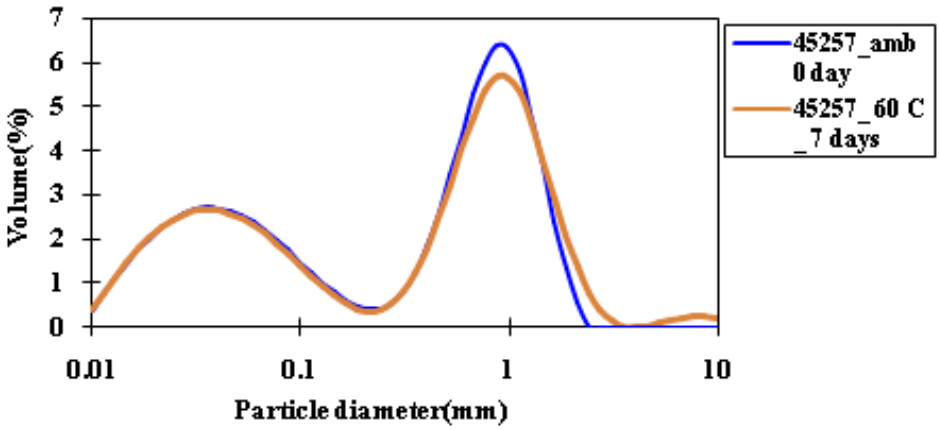


Figure 3.14: Particle size distributions of *Acacia nilotica* var. *adstringens* emulsion samples (freshly and 7days at 60° C).

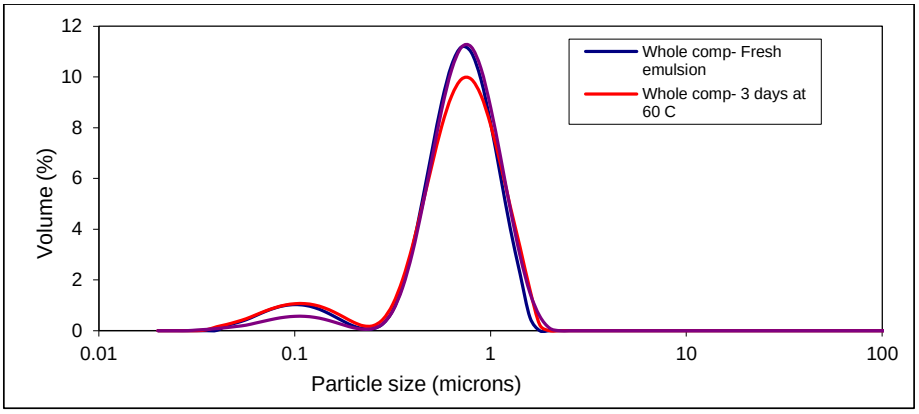


Figure 3.15: Particle size distributions of *Acacia nilotica* var. *nilotica* emulsion samples at different conditions Satti (2011).

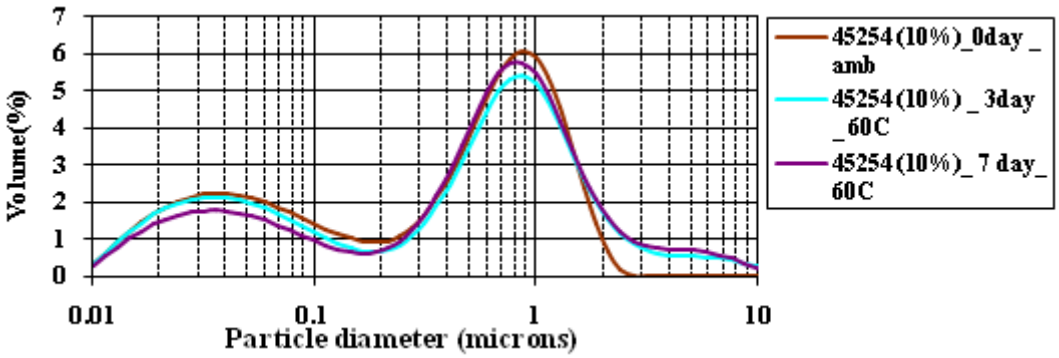


Figure 3.16: Particle size distributions of *Acacia nilotica* var. *tomentosa* emulsion samples at different conditions Seifeldawla (2018).

3.14 Rheological properties

3.14.1 Shear flow viscosity

The effect of shear rate on viscosity, at 25^{o}C, for *Acacia nilotica* var. *adstringens* gum solutions at different concentrations (25% and 50%) is shown in Figure (3.17). The Figure illustrates the viscosity- shear rate at 25% concentration; the flow curves at increasing shear rates showed that the apparent viscosity ($\eta 0$, Pa.s) decreased slightly as the shear rate(γ , s^{-1}) increased, a plateau was reached for $\gamma > 1s^{-1}$. A Newtonian low-shear plateau (the so-called zero-shear viscosity) was observed even at shear rates as low as $0.1s^{-1}$. At intermediate shear rates (~ 1 s^{-1}) a trend to a high-shear Newtonian plateau was observed. The flow curve show a shear-thinning at lower shear rates, and a viscosity plateau (Newtonian behavior) at higher

shear rates. Similar rheological observations already reported for gum arabic (Williams *et al*., 1990, Mothé *et al*., 1999, Sanchez *et al*., 2002), *A.nilotica* var.*nilotica* gum (Satti, 2011) and for *A.nilotica* var.*tomentosa* gum (Seifeldawla, 2018). The flow behaviour of gum solutions is related to the colloidal nature, the average particle size and the size distribution. The extent of the shear thinning seems to be reduced at higher concentrations. That may be regarded as arising from modifications in the macromolecular organization of the solution as the shear rate changes.

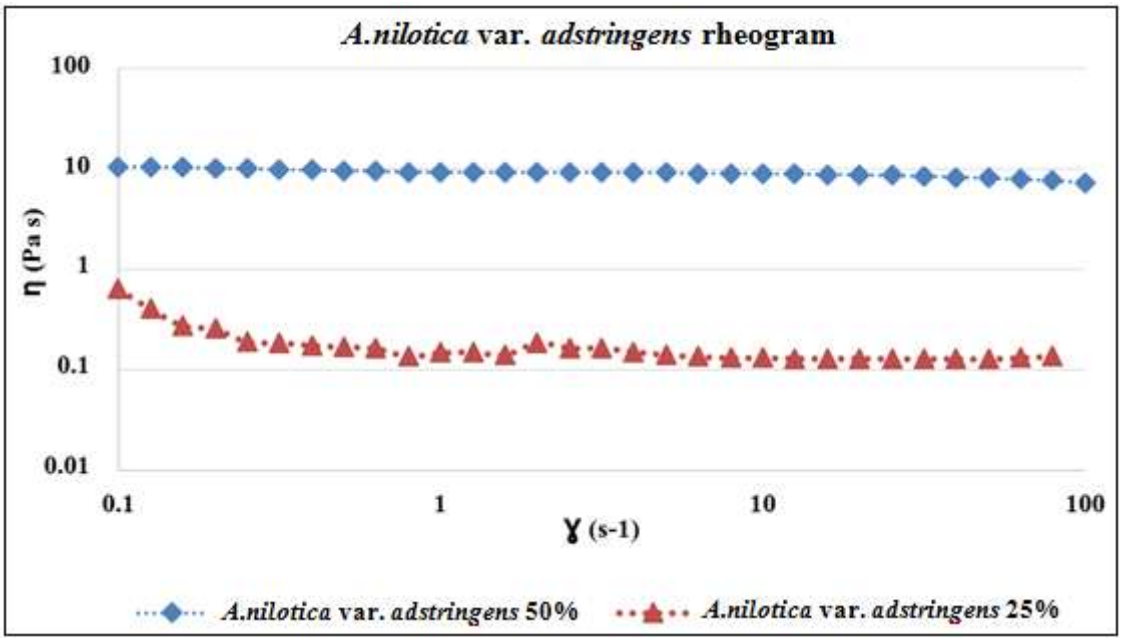


Fig 3.17 Viscosity-Shear rate profile of *Acacia nilotica* var. *adstringens* gum solution at 25% and50% concentration.

At concentrations of 50% solutions, gum exhibit Newtonian flow. Therefore, the rheological behavior of gum solutions approach Newtonian character as the gum concentration increases, Figure (3.17). The presence of a large number of high molecular weight molecules increases the resistance to flow which, in turn, increases the apparent viscosity of the gum solutions. Newtonian behaviors were expected as a result of the relatively compact conformation of branched gum (Tanaka *et al*., 2006). This result is similar to that obtained by Satti (2011) for *A.nilotica* var. *nilotica* gum Fig (3. 18) and Seifeldawla (2018) for *A. nilotica* var. *tomentosa* gum Fig (3.19).

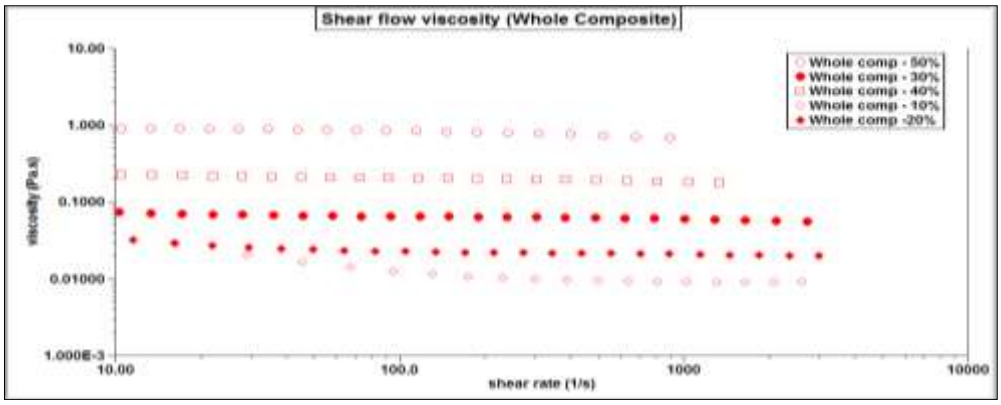


Figure 3.18: Viscosity-Shear rate profile of *Acacia nilotica* var. *nilotica* gum solutions at 10, 20, 30, 40 and 50 % concentration (Whole comp).

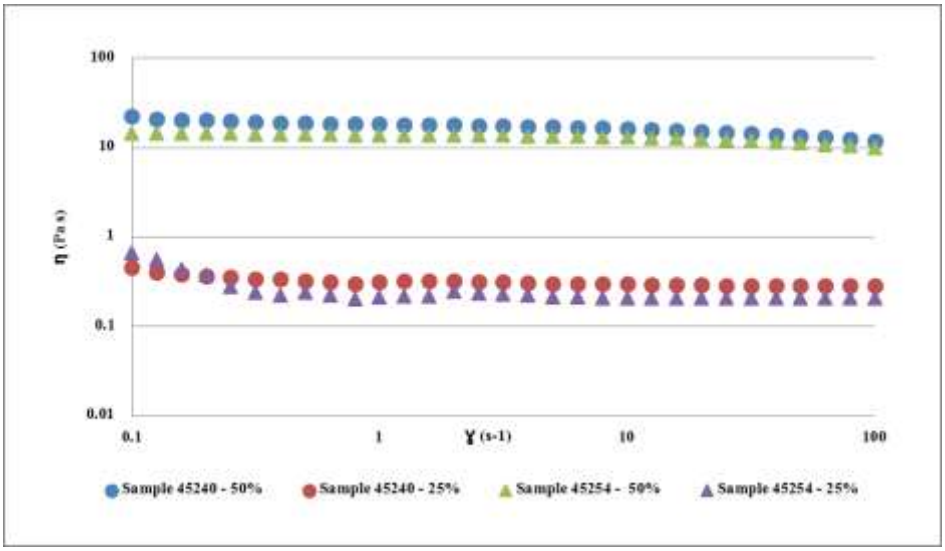


Figure 3.19: Viscosity-Shear rate profile of *Acacia nilotica* var. *tomentosa* gum solutions at 25 and 50 % concentration (Whole comp).

The effect of concentration on viscosity has also been observed for gum exudates from *A. senegal* (Mothé *et al*., 1999) and *A. tortuosa* (Munoz *et al*., 2007). In gum arabic, the fact that solutions of high concentration (50%) and low viscosity can be obtained has led to its extensive use in confectionery products. *Acacia nilotica* gum solutions exhibit a high viscosity at low shear, shear thinning properties at higher shear rates and eventually a high-shear rate Newtonian region similar to that found for gum arabic. The breakdown of microstructures can be enhanced with increasing the applied shear rate while the relevant rebuildup occurs with the decrease of shear rate. Both *A. senegal* and *A. seyal* exhibit a Newtonian behaviour at low and moderate temperature (Hassan, 2000). At 60°C *A. seyal* showed marked increase in the viscosity at low shear rate region (~1 s^{-1}). The viscosity

decrease steadily with increasing shear rate till the shear rate reaches a value of $100s^{-1}$, and a typical Newtonian behaviour started to dominate. The same behaviour was shown by *A. senegal* but at a higher temperature (70°C). Figures (3.20 and 3.21) show that *A.nilotica* var. *adstringens* gum exhibit Newtonian flow behavior at different concentration.

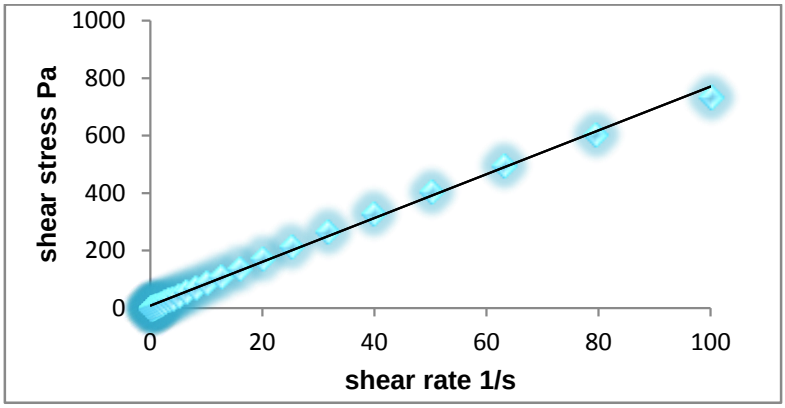


Figure 3.20: The shear rate vs. shear stress of *A.nilotica* var. *adstringens* gum solution at 50% concentration.

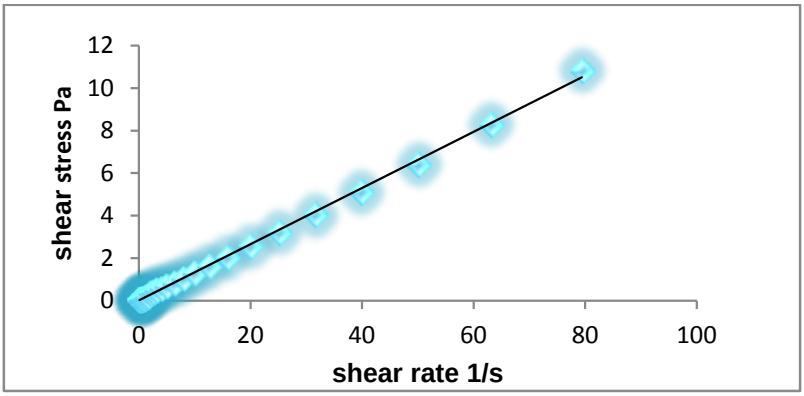


Figure 3.21: The shear rate vs. shear stress of *A.nilotica* var. *adstringens* gum solution at 25% concentration.

3.14.2 Dynamic rheological behavior

Viscoelastic properties of *A. nilotica* var. *adstringens* gum solution at 50% concentration were determined using oscillatory testing. Mechanical spectra of solutions revealed a typical liquid-like behaviour (Figure 3.22). The loss modulus (G″) was higher than the storage modulus (G′). G″ is dominating G′ throughout the tested oscillatory deformation regime, and

hence the viscous behavior of the gum dominates the elastic behavior. Therefor the energy used to deform the material is dissipated viscously and the sample exhibits liquid-like behaviour.

The moduli showed less frequency dependence at lower frequency range and relatively higher frequency dependence at higher frequency range.

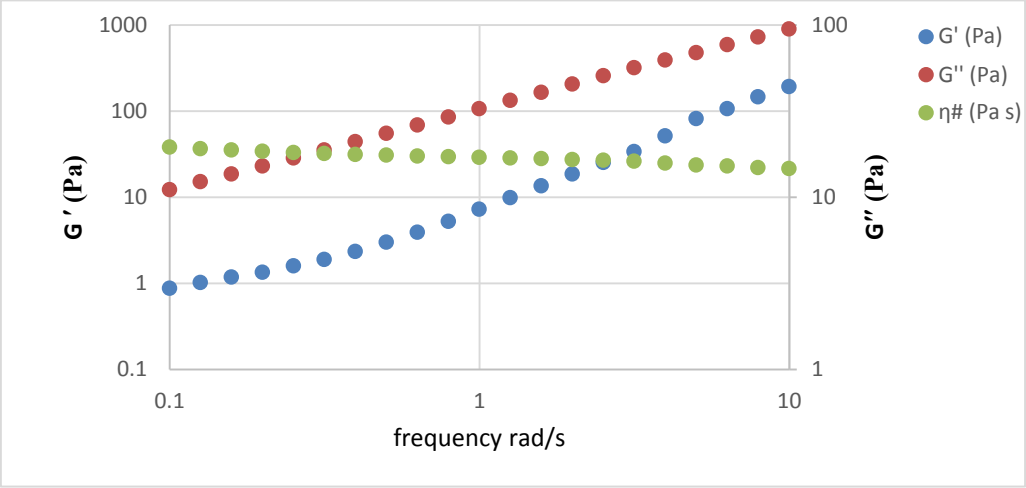


Figure 3.22: The dynamic rheological behaviour of *Acacia nilotica* var. *adstringens* gum solution at 50% concentration

Hassan (2000) studied the effect of temperature in the dynamic rheological behaviour of both *A. senegal* and *A. seyal* and he reported that *A. seyal* gum at temperatures less than or equal to 60°C reflects a viscous behaviour, where G″ is greater than G′, i.e. energy was dissipated, irrecoverably, as frictional heat. At temperature equal to 70°C the behaviour became elastic at the low frequency region. Also *A. senegal* at temperatures less than or equal to 60°C shows viscous behaviour, but at 70°C *A. senegal* had a completely different behaviour. The gain modulus G′ is greater than G″. At high temperature the molecular motion increases hence enhancing the probability of molecular interaction. High temperatures facilitate hydrophobic interactions a matter that might contribute positively to stabilization of molecular aggregation or networks that might have been formed due to entanglements.

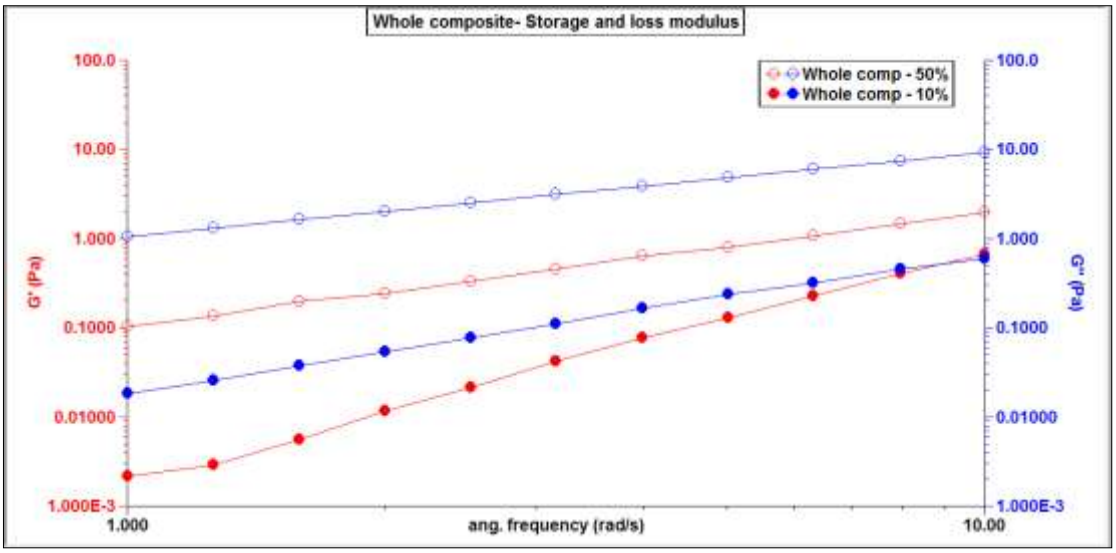


Figure 3.23: The dynamic rheological behaviour of *Acacia nilotica* var. *nilotica* gum solutions at 10 and 50% concentration (whole composite) (Satti, 2011).

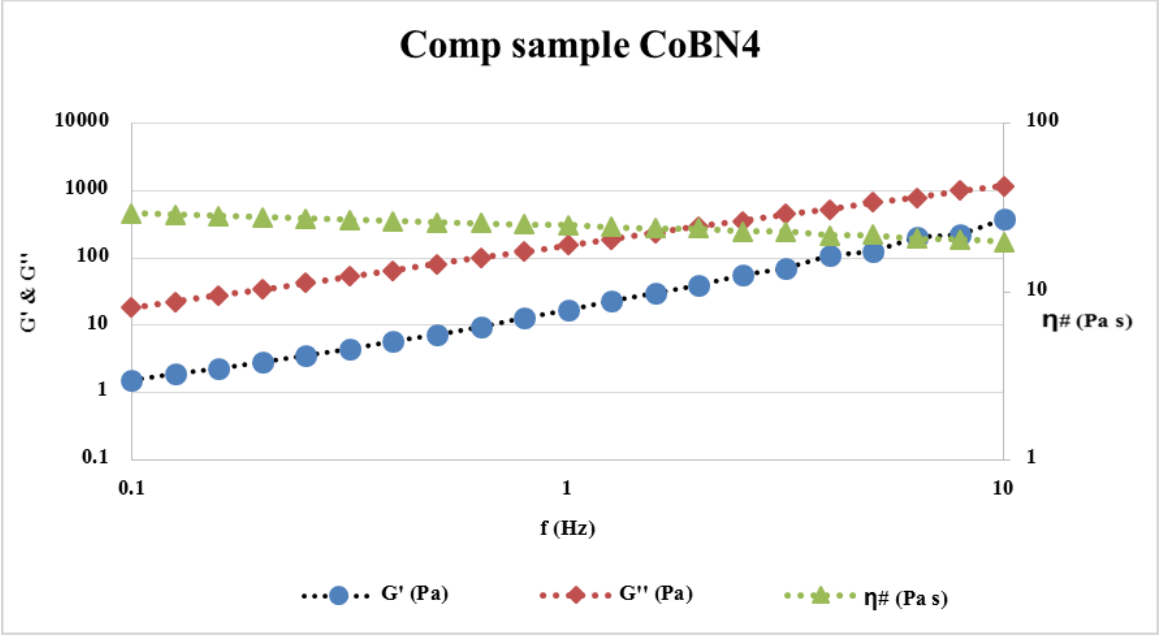


Figure 3.24: The dynamic rheological behaviour of *Acacia nilotica* var. *tomentosa* gum solution at 50% concentration (Seifeldawla, 2018).

3.15 Conclusion

Composite samples of *A. nilotica* var. *adstringens* gum were evaluated to characterize their physicochemical properties. The data obtained that *A. nilotica* var. *adstringens* gum belongs to gummiferae series. These properties can be summarizing as follow:

A. nilotica var. *adstringens* gum contains a lower proportion of nitrogen and hence lower protein contents compared to *Acacia senegal*. It possessed positive optical rotation in constrast to *A. senegal* which has negative optical rotation. Also *A.nilotica* var. *adstringens* gum has lower viscosity, lower glucouronic acid contents, higer arabinose and higer molecular weight compared to *A. senegal* and *A. seyal*.

Different physicochemical properties of samples tested agree with previously reported studies of *A.nilotica* var. *nilotica* and *A. nilotica* var. *tomentosa* gum.

A.nilotica var. *adstringens* gum produced emulsions having very good emulsifying stability compared to *A. senegal*, although larger droplet size and lower protein content than *A.senegal* gum.

A.nilotica var. *adstringens* gum showed shear thinning flow behavior under low rate and Newtonian behavior at high shear rate and high concentration and it revealed a typical liquid-like behavior.

3.16 Suggestion for further work

- For better understanding of the properties of *Acacia nilotica* var. *adstringens* gum in order to improve its quality and widen its applications in food and other industries, further research should carried to study the toxicity in order to be used in food industry.
- Study the thermodynamic properties of gum solutions to understand the interaction of gum molecule with solvents and its behavior in real food and pharmaceutical system.

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