Sudan University of Science and Technology

College of Graduate Studies

Physicochemical and Functional Properties of Glucuronic acid and Potassium Glucuronate from *Acacia senegal* var. *senegal* Gum

الخواص الفيزيوكيميائية والوظيفية لحمض الجلوكيورنيك و جلوكويورنات البوتاسيوم من الصمغ العربي

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الآيه

قال الله تعالى :

(يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات والله بما تعملون خبير)

صدق الله العظيم

(سورة المجادلة الآية 11)

Dedication

I dedicate this research:

To all members of my family.

To my marvelous friends and colleagues.

To all persons who gave me care, support and love.

Acknowledgment

My special praise and thanks be to Allah, the Almighty, most Gracious and most Merciful who gave me the health, strength and patience to conduct this research.

I would like to express my deep gratitude and thanks to my supervisor Dr. Mohammed Elmubark Osman for his guidance, and assistance during this study.

Special thanks and acknowledgments are also due to my family and friends for their support and help.

ABSTRACT

Gum arabic (*Acacia senegal*) from Al Gadaref was used in this study. Physical and chemical properties such as moisture content, Total ash, pH value, intrinsic viscosity, Molecular weight, specific optical rotation, nitrogen and protein, acid equivalent weight, total glucuronic acid, and cationic composition were determined for gum Arabic. The values of moisture and ash were found to be 8.4 % and 3.2 % respectively, while the specific rotation value of the sample was found to be-27.5. The intrinsic viscosity of sample was found to be 15.3ml/g, while the calculated molecular weight value from Mark-Houwink equation was $4.85x10^5$. The pH value was found to be 4.6. while nitrogen content was 0.227%. The calculated protein value from the conversion factor of 6.6 was found to be 1.5 %. The values of acid equivalent weight and percentage of total glucuronic acid were found to be 1700.7 and 11.4 % respectively. Results indicate that potassium is the major element present in *Acacia senegal* gum (1952.490ppm) when compared with calcium, sodium and magnesium.

Glucuronic acid was prepared from *Acacia senegal* gum by the removal of metal ions using ion exchange chromatography. In addition Potassium glucuronate was prepared using glucuronic acid by titrating with potassium hydroxide solution. Glucuronic acid and potassium glucuronate gave pH values of 2.7 and 6.8 respectively. The values of moisture content were found to be 6.8% and 7.1% respectively, while the intrinsic viscosities were 10.8 and 12.9ml/g. The values of total ash were found to be 0.3% and 7.7% respectively. The specific optical rotation value of glucuronic acid and potassium glucuronate were found to be -20, -25 respectively. In addition the stability of *Acacia senegal*, glucuronic acid and potassium glucuronate emulsions with regard to type of oil (sunflower and groundnut) were also investigated. Results indicate that emulsion stability is significantly affected by the type of emulsifier and the type of oil used. *Acacia senegal* gum and potassium glucuronate formed more stable emulsions than glucuronic acid. Sunflower oil gave the most stable emulsions than groundnut oil.

المستخلص

في هذه الدراسة استخدم الصمغ العربي (الاكاشيا سنغال) من القضارف.تم تحديد الخواص الفيزيائية والكيميائية مثل محتوى الرطوبة ، الرماد الكلي ، قيمة الاس الهيدروجيني ، اللزوجة الضمنية ، الوزن الجزيئي ، الدوران النوعي ، النتروجين و البروتين ، الوزن المكافئ الحامضي ، حامض الجلوكيورنيك الكلي وتركيب المعادن للصمغ العربي. وجد ان قِيَم الرطوبة والرماد 8.4% و3.2% على التوالي،. بينما وجد ان قيمة الدوران النوعي للعينه 27.5-. اللزوجة الضمنية للعينه كانت 15.3مل/جرام، بينما قيمةِ الوزن الجزيئي المحسوبه من معادلة مارك - هيونك كانت $4.85x10^5$. كما وجد ان قيمة الاس الهيدروجيني 4.6. بينما محتوى النتروجين0.227 %. قيمة البروتين المحسوبه من معامل التحويل 6.6 كانت 1.5 %. قيم الوزن المكافئ الحامضي والنسبة المئوية لحامض الجلوكيورنيك الكلي 1700.7،11.4 % على التوالي. تشير النتائج ان البوتاسيوم هو العنصر الرئيسي الموجود في صمغ الاكاشيا سنغال (1952.490 جزء في المليون) عندما قورن مع الكالسيوم، الصوديوم والماغنسيوم.

حضرحامض الجلوكيورنيك من صمغ الاكاشيا سنغال بإزالة الآيونات المعدنية باستعمال كروموتغرافيا التبادل الآيوني. بالاضافه الي ذلك حضر جلوكويورنات البوتاسيوم بإستعمال حامض الجلوكيورنيك بمعايرتة مع محلول هيدروكسيد البوتاسيوم. أعطى حامض الجلوكيورنيك وجلوكويورنات البوتاسيوم قيم اس هيدروجيني 6.8 و 2.7 على التوالي. وجد ان قيم محتوى الرطوبة 6.8% و 7.1%على التوالي، بينما قيم الرماد كانت 0.3% و 7.7% على التوالي.كما وجد ان اللزوجة 10.8،12.9 مل /جرام. وجد ان قيمة الدوران الضوئي النوعي لحامض الجلوكيورنيك وجلوكويورنات البوتاسيوم -20 و - 25على التوالي. بالاضافه الي ذلك تمت دراسة إستقرارية مستحلبات اكاشيا سنغال، حامض الجلوكيورنيك وجلوكويورنات البوتاسيوم باعتبار نوع الزيت (عباد الشمس والفول السوداني). اوضحت النتائج أن إستقرارية المستحلب يتاثر بشكل ملحوظ بنوع عامل الاستحلاب ونوع الزيت المستخدم. صمغ الاكاشيا سنغال، وجلوكويورنات البوتاسيوم تكون مستحلبات اكثر استقرارا من حامض الجلوكيورنيك.أعطى زيت عباد الشمس مستحلب اكثر إستقراراً من زيت الفول السوداني.

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Introduction and Literature Review

1. Introduction

1.1. Gum arabic

The oldest and best known of all natural gums is gum arabic obtained from *Acacia senegal*, var. *senegal*. It is known as an important article of commerce for about 4000 years ago. The Joint Expert Committee for Food Additives (JECFA) defined Gum Arabic as the dried exudation obtained from the stem and branches of *Acacia senegal*(*L*) Willdenow or related species of *Acacia* (FAO,1982). There are more than 1000 species of *Acacia*, but, the gum from *Acacia senegal* is, perhaps, the most valuable and, widely, used species of natural plant gums (Islam *et al*., 1992). Gum Arabic 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 polysaccharide acid (arabic acid).However, other heavy elements such as Zn, Al, Cd, Cu, Cr, Pb, and Co may also be present but in very small quantities (Islam *et al*., 2004).
An *FAO (JECFA*, 1990*)* specification existed for Gum Arabic intended for use as a food additive; in the United States, a Food Chemicals Codex specification exists. For pharmaceutical use, Gum Arabic appears in many pharmacopoeias, including the British Pharmacopoeia. The *JECFA* (1990) specification has undergone a number of revisions over the years. The present one specified limits on such parameters as loss on drying, ash, acid-insoluble matter, arsenic, lead and heavy metals. A departure of the present specification from earlier ones (other than a modified definition) is the inclusion of limits on optical rotation and nitrogen content. Their inclusions, and the numerical limits, are designed to ensure that as far as possible, only gum from *A. senegal* or closely related species is able to satisfy the requirements (AIPG).

1.2. Food Emulsions

The purpose of emulsion science in food industry is to develop food quality and production techniques by benefiting from emulsion principles. Emulsions take place partially or completely in the structures of many natural and processed foods or some foods are already emulsified in certain stages of production (McClements, 2005). Foods like milk, cream, butter, margarine, juice, soup, cake, pastry, mayonnaise, cream liqueur, coffee creamer, sauce and ice cream may be shown as examples to emulsion-type products (Dickinson and Stainsby,

1982; Krog et al., 1983; Dickinson, 1992; Swaisgood, 1996; Friberg and Larsson, 1997; Charcosset, 2009). Furthermore, many meat products depend on the presence of emulsions for their properties, as does bread dough, although in both cases the emulsion structures can be extremely complex (Dalgleish, 2004). Emulsion based food products have important differences in terms of physicochemical and organoleptic characteristics like appearance, flavour, texture, taste and shelf life. For example, milk is a white fluid with low viscosity, strawberry yogurt is a pink viscoelastic gel and margarine is a yellow semi-solid. Production of an emulsion based food product having specific quality characteristics depends considerably, on the selection of most appropriate raw materials (for example; water, oil, emulsifying agent, thickening agent, minerals, acids, bases, vitamins, aromas, colorants, etc.) and process conditions (for example mixing, homogenization, pasteurization, sterilization, etc.) (McClements, 2005).

2. Literature Review:

2.1. Gum Arabic:

Gums are dried, gummy exudation obtaineds from various species of *Acacia* trees of the Leguminous family (FAO, 2007). The JECFA defined Gum Arabic as the dried exudate from the trunk and branches of *Acacia senegal* or *Acacia seyal* of the family Leguminous (Rahim *et al.,* 2007) *Acacia* trees belong to the botanical family of Leguminous, predominantly species of the groups Fabales and Gummiferae. There are more than one thousand species of *Acacia*, out of which only three produce gums of commercial value with different properties. The only species producing sap eligible for the name *Acacia* gum or Gum Arabic are *Acacia senegal* and *Acacia seyal* (FAO,2007).

2.1.1. The gum arabic belt

The gum belt referre to a broad band, situated at a latitude between 12° and 16° North, stretching across sub-Saharan Africa, from Mauritania in the West, through Senegal and Mali, Burkina Faso, Niger , Northern Nigeria to Sudan, Eritrea, Ethiopia, Kenya, Somalia and Northern Uganda in the East. Production of Gum arabic is concentrated in the "gum belt", an area of central Sudan roughly between latitudes 100˚ and 140˚ north. Two areas outside these borders are in the north east (Faw-Gedaref-Kassala) and in the south east along the Blue Nile/Upper Nile border.

It is estimated to cover 520.000 square kilometers, roughly one fifth of Sudan's total area. It is spanned over 12 states:-Western Darfur, North. Darfur, South. Darfur, North. Kordofan, Western. Kordofan, South. Kordofan, White Nile, Upper Nile, Jonglie, Sennar, Blue Nile

and Gedaref. The sandy plains are in the first seven states and the clay plains are in the latter five states (FAO, 2007).

2.1.2. Chemical structure of plant gums

Gum nodules are polysaccharide material of complex nature usually contaminated with impurities such as bark fragments, entrapped dust and insects. Inert pertinacious material and a few amounts of terpenoi resins can also be present. Gums are polyuronides; the uronic acid residues may carry acetyl o methyl groups and, generally, occur at least in part as methyl groups and generally occur, at least in part, as metallic salts. Figure 1.1: Structure of polysaccharide of *A .senegal* (Street and Anderson,1983).

The hexose residues are present in the pyranose configuration, while the pentose residues occur in the furanose (Stephen *et al.,* 1955 and 1957) Beside the foregoing other gums, have been studied; *khaya senegalese* gum contains galactose, rhamnose and probably 4-O-methyl, D-glucuronic acid and galactouronic acid (Aspinal *et al.,* 1956). *Sterculia termentosa* gum contains rhamnose, galactose and probably galacturonic acid, Olibanum gum (*Boswellia carterii*) was found to be of an arabino-galactan and a polysaccharide containing galactose and galactouronic acid (Elkhatem *et al.,* 1956). It was noted that the gum was very heterogeneous and it has been 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 unites, in addition to distribution in molecular weight (Lewis and Smith, 1957; Dermyn, 1962 and Stoddart, 1966). According to Philips (1988) and Williams *et al*., (1989), fractionation by hydrophilic affinity chromatography revealed that *Acacia senegal* gum consists of at least three distinct components. Fraction 1 AG (arabino galactan), fraction 2 AGP (arabino galactan-protein) and fraction 3 GP (galactoprotein). But even those contain a range of different molecular weight components revealing the polydiverse nature of the gum (Osman *et al*., 1994). Fraction 1 containing 88% of the total has only small amount of protein content. Fraction 2 represents 10% of the total and had 12% protein content. Fraction 3 resembles 1.24% of the total but contains almost 50% of protein AGP is responsible for the emulsifying properties of gum arabic (Williams *et al*., 1989, and Phillips, 1988). Figure 1.2: Hypothetical structure of arabinoglactan-protein (AGP) obtained from gum arabic (Street and Anderson,1983).

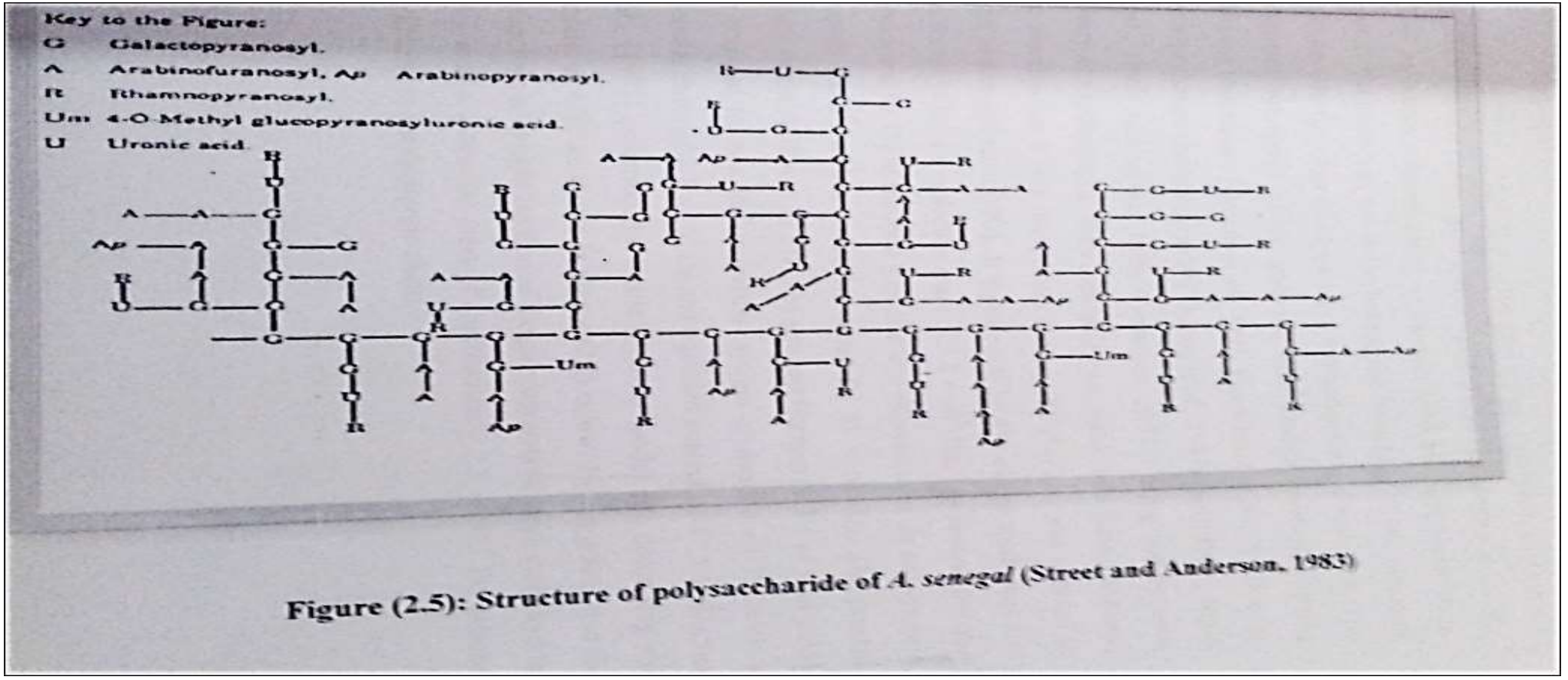

Figure (2.5): Structure of polysaccharide of *A. senegal* (Street and Anderson, 1983)

Fig 1.1: Structure of polysaccharide of *A. senegal* (Street and Anderson, 1983)

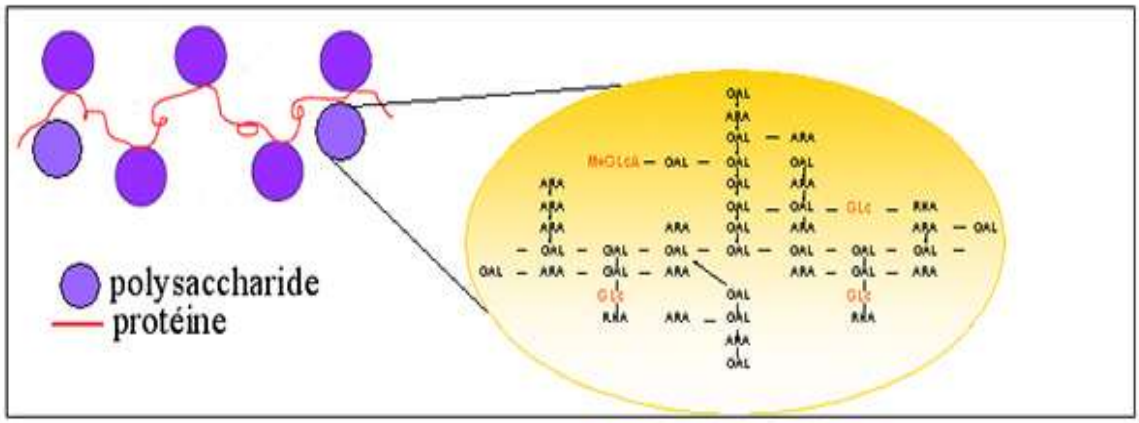

Fig 1.2: Hypothetical structure of arabinoglactan-protein (AGP) obtained from gum arabic

No mention has been made to detailed comparison between the structures of gums from different species of trees, but is believed that D-galactose and uronic acid residues generally constitute the backbone of the polysaccharide with 1-3 and 1-6 linkages predominating side chain are characterized by the presence of D-xylopyranose, L-arabinose, and L-arabino-furanose linkage (Williams, 1989, and Phillips, 1988).

2.1.3. Biosynthesis of the gum

The gum is produced by trees aged between three and thirty years (Blunt, 1926) and especially so if they are in an unhealthy condition. Moreover it is well established that natural factors such as poor soil, lack of moisture and hot temperature, all of which tend to lessen the vitality of vegetation, generally improve the gum yield from Acacia trees (Mantell, 1954).

There is no clear evidence so far to describe the biosynthesis of the gum (gummosis). However it has been proposed by Greig (1902) that gummosis is a pathological condition resulting from a microbial (bacterial or fungal) infection of the tree. He reported the isolation of a bacterium in pure culture from an *Acacia binervata* tree which he named Bacterium Acacia. The bacterium was reported to form a gummy substance having similar properties to the natural exudates. Greigs findings have been supported by similar experiments on *Acacia senegal* according to Ghosh and Purkayastha (1962). Blunt (1926) also held similar views as the trees produce the gum when in an unhealthy condition which is congenial to bacterial activity. Gummosis, however, is considered by Malcom (1936) to be a normal metabolic process of the tree, and the gum is produced in response to physiological disturbances induced by drought. Differences of opinions also exist

(Anderson and Dea, 1968) on whether the exudate is formed on site at the surface of the trunk and branches of the tree, or generated internally and transported to the site of exudation. The gum is believed to act as an efficient natural "sponge" which seals off wounds thereby forming a protective coating to prevent complete local dehydration of the plant tissue (Anderson and Dea, 1971). Histochemical investigations of some specimens from *Acacia senegal* trees have shown that gum ducts develop in the phloem parenchymatous cells adjacent to the cambium (Ghosh and Purkayastha, 1962). They have also suggested that the cells of the inner phloem take part in the formation of a gum cyst and that the development of the cyst is preceded by wide spread changes in the xylem and phloem induced by physiological disturbances in the tree due to some pathological condition caused by bacterial activity. *Khalid et al.,* (1988), working with gum from *Acacia senegal* have isolated five genera of moulds, namely *Aspergillus, Penicillium, Rhizopus, Gilocladium* and *Cladosporium.* They also showed that Sudanese gum arabic is free from pathogenic microflora e.g. *Salmonella* and *Coliform* species, and ruled out the role of *Gladospronium gladospordides* in the initiation of gummosis. Joseleau and Ullmann (1983; 1990) have investigated the carbohydrate composition of both water, and alkali soluble extracts obtained from the bark, cambium, and xylem of gummiferous and non-gummiferous (control) branches from an *Acacia senegal* tree. They concluded that gummosis is restricted to the cambial zone, and is directly related to starch metabolism.

2.1.4. Collection and processing of gum arabic

Although natural exudates are sometimes harvested, virtually all exudate gums are tapped from the tree. When *Acacia* trees lose their leaves and become dormant at the beginning of the dry season , usually by the end of October or beginning of November, superficial incisions are made in the branches and bands of bark when are stripped off. After 5 weeks, gum is manually collected as partially dried tears or nodules. This collection is repeated at 15-day intervals for up to five or six collections in total, depending on the weather conditions and the health of the tree (Imeson, 1992). After, the collection, gum is cleaned and graded. This is, traditionally, done by women who, manually, sort the gum according to the size of the lumps and remove foreign matter (FAO, 1995). Since the 1990s, cleaning has also been performed mechanically using conveyor belts and sieving machines. In Sudan, the gum from *Acacia senegal* (hashab) is presented in various grades, Table 2.1. Since 1995; gum from *Acacia seyal* (talha) has been divided into three grades: super, standard clean, and Siftings (FAO, 1995). Grade 1 is gum obtained from *Acacia senegal*

and comparable to cleaned hashab. Grade 2 is produced by other *Acacia* species, such as *Acacia seyal* and *Acacia sieberana.* Grade 3 may contain gum from species other than *Acacia*, like *Cumbretum* and *Albizia*. After collection the gum can be further processed into kibbled and powdered forms. Kibbling is a mechanical process which breaks up large lumps into smaller granules with a more uniform size distribution and facilitates the dissolution of the gum in water. Even better solubility characteristics are obtained with powdered gum, which is, usually, produced by dissolving the gum in water, removing impurities by filtration or centrifugation and spray-drying.

Table 2.1: Commercial grades of *Acacia senegal* gum from Sudan (Islam *et al*., 1997).

Grade	Description
Hand-picked Selected	cleanest and largest pieces with the lightest color. The most expensive grade.
Cleaned and Sifted	The material which remains after hand-picked selected and siftings are removed. This grade comprises whole and broken lumps with a pale to dark amber color.
Cleaned	The standard grade with a light to dark amber color. It contains siftings but the dust is removed.
Siftings	The residue formed by sorting the above, more choice grades. This grade contains a proportion of sand, dirt and bark.
Dust	This grade is collected after the cleaning process and comprises very fine particles of gum,sand and dirt.
Red	Dark red gum particles removed from The lumps.

2.1.5. Physiochemical properties of gum arabic

The physical properties of the natural gums are most important in determining their commercial value and their use. These properties vary with the gum 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 seasons of the year (Hirst and Jones, 1958). The physical properties may also be affected by the age of the tree and treatment of the gum after collection such as washing, drying, sun bleaching and storage temperature.

2.1.5.1. Solubility

Gum arabic is unique among the natural hydrocolloids because of its extremely high solubility in water and can yield solutions of up to 60% concentration and it is truly soluble in cold water, other gums are either insoluble in cold water or form colloidal suspensions "not true solutions"(G.A.C, 1993).

2.1.5.2. Moisture content (%)

Moisture content involves measuring the weight lost due to the evaporation of water (Person, 1970). It is helps to determine the dry weight of the gum (FAO, 1988).

2.1.5.3. Total ash content (%)

Ash content is a measure of inorganic residue remaining after removal of organic matter by burning. The inorganic residue exists as elements explaining that, the type of the soil (clay or sand) affect the ash content significantly (FAO, 1988).

2.1.5.4. Nitrogen and protein content (%)

Protein content in gum arabic has effects on emulsifying behavior of gum arabic and the best emulsion capacity and stability is found in gums with highest nitrogen content (Randall *et. al*., 1988, Dickinson, 1992).

Anderson (1986) found that the average nitrogen content for commercial *Acacia senegal* gum formulations to be 0.37%. Investigations of protein in *Acacia senegal* gum have been carried out by Akiyama *et al.,* 1984. They reported that gum arabic contained 2.0% protein and they established that amino acid of gum arabic is rich in hydroxyproline and serine. Anderson *et al.,* (1985) described gum arabic as a proteinaceous polysaccharide with a protein content ranging from 1.5 to 3.0%. They concluded that the variation was mainly due to different localities. and reported the value of 0.23-0.58% nitrogen for commercial formulations. Osman (1998) reported 0.33- 0.36% nitrogen (2.14-2.16% protein) for *Acacia senegal* gum and Jurasek *et al.,* (1993) in a chemo metric study for different *Acacia* species

reported 0.27-038% nitrogen for commercial samples of gum arabic from Sudan. Awad Elkarium (1994) reported that the average nitrogen contents of different commercial grades are around 0.28%. Karamalla *et al.*, (1998) reported that the average nitrogen content of different commercial grades is around 0.33%.

2.1.5.5. Specific optical rotation

Specific optical rotation (α) is a property of chiral chemical compounds. It is defined as the change in orientation of monochromatic plane-polarized light, as the light passes through a sample of a compound in solution. Compounds which rotate light clockwise are said to be dextrorotary, and correspond with positive specific optical rotation values, while compounds which rotate light anticlockwise are said to be levorotary, and correspond with negative values. If a compound is able to rotate plane-polarized light, it is said to be "optically active". Specific optical rotation is an intensive property, distinguishing it from the more general phenomenon of optical rotation.The observed rotation (α) of a sample of a compound can be used to quantify the enantiomeric excess of that compound, provided that the specific rotation (α) for the enantiopure compound is known. The variance of specific rotation with wavelength (a phenomenon known as optical rotatory dispersion)can be used to find the absolute configuration of a molecule (Biswas *et al.*, 2000).

Karamalla *et al.*, (1998) reported a specific optical rotation for 789 authentic *Acacia senegal* gum samples, between -26^{o} to -34^{o}.Vavdevelde and Fenyo (1985) reported specific optical rotation of *Acacia senegal* gum to be ranging between -29^{o} to -34.4^{o}. Jurasek *et al*; (1993) reported the specific optical rotation of *Acacia senegal* gum to be ranging from -20^{o} to -32^{o}. FAO (1990) reported the value of specific optical rotation to be in the range of -26^{o} to -34^{o}. Abdelrahman (2008) reported the average value of optical rotation of *Acacia senegal* gum to be -31.5$^{\mathbf{o}}$.

2.1.5.6. Viscosity

The viscosity of liquid is its resistance to shearing, to stirring or to flow through a capillary tube (Bancraft, 1932). 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 Dea, 1969). The viscosity of a solution may have a complicated variation with composition, due to the possibility of hydrogen bonding among the solute and solvent molecules (Pimentel and McClements, 1960). More hydroxyl groups makes high viscosities, because a network of hydrogen bonds is formed

between the molecules, this network extends throughout the liquid, thus making flow difficult. The viscosity of gum solutions is inversely proportional to temperature. They also found that the viscosity of gum arabic solutions changes with pH, but they found a maximum viscosity at pH 6-7. Viscosity can be explained in different terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic; it is also represented as kinematics or dynamic viscosity.

Duvallet *et al*., (1989) reported that the intrinsic viscosity of *Acacia senegal* has a value of 21.8cm^3 g^{-1}. Jurasek *et al*., (1993) found that the intrinsic viscosity ranged between 13.4 – 23.1cm^3 g^{-1} for *Acacia senegal*, Anderson (1977) reported a value of 13.4 cm^3 g^{-1}intrinsic viscosity for *Acacia senegal*.

Table 2.2: Analytical data for authentic *Acacia senegal var senegal* gum samples collected in season 1994/1995 (Karamalla *et al*., 1998)

Variable	No. of samples	Minimum	Maximum	Mean
Moisture (%)	803	8.1	14.05	10.75
Ash (%)	731	2.75	5.25	3.77
Nitrogen (%)	642	0.225	0.425	0.328
Specific optical rotation	789	-23	-39	-31.3
pH	755	4.3	5.1	4.66
Equivalent weight	115	1136	1875	1436
Uronic acid anhydride (%)	115	10.34	23.32	13.71
Intrinsic viscosity	94	1	73	16.44

2.1.5.7. Molecular weight

The molecular weight of the polymers can be determined from physical measurement or by application of chemical methods. The applications of chemical methods require that the structure of the polymer should contain a well known number of functional groups per molecule and they invariably occur as end groups. The end group analysis method gives an approximately number of molecules in a given weight of sample; they yield the average number of molecules for polymeric materials. This method becomes insensitive at high molecular weight, as the fraction of end groups becomes too small to be measured with precision (Meyer, 1971). This is due to the fact that fraudulent sources of the end groups

not considered in the assumed reaction mechanism steadily become consequential as the molecular weight increases and the number of end groups diminishes to such an extent their quantities determination is not feasible. Those reactions confine frequent application of chemical methods to condensation polymers with average molecular weight seldom exceeding 2.5 $\times 10^3$ (Flory, 1953). Physical methods frequently used for establishing polymer molecular weight are osmometry, polymer viscosity, measurement of coefficient of diffusion, ultra centrifugation and light scattering. One of the most recent advanced methods is light scattering (LS), which provides an absolute method for polymer molecular weight and size measurement. LS is rapid, accurate and requires a small amount of sample. The molecular weight of gums varies greatly in values due to gum heterogeneity as well as variation in techniques used to separate, purify and determine the molecular weight. A 3.0×10^3 was reported by Saverbon (1953) using centrifugal method. Using the light scattering technique gave higher values Veil and Eggenberger (1954) reported a Mw =1.0×106; Mukherjee and Deb (1962) reported Mw up to 5.8 × 105 and Fenyo (1988) reported a range of 4.0 × 106 to 2.2 × 106.Recently GPC coupled on line to multi angle laser light scattering (MALLS) has been demonstrated to be a very powerful method for characterizing highly polydisperse polymer systems and the molecular weight of *A. senegal* gum was found to be equivalent to 5.4×10^5 (Picton *et al*., 2000).

2.1.5.8. Acidity and pH measurements

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. Arabic acid substance is the major component of commercial gum Arabic and when decomposed, it gives arabinose, so that gum arabic is called arabic acid.

Karamalla (1965) reported a pH values of 4.42 for *Acacia senegal* gum while he recorded a value of 4.74 for *Acacia seyal var. fistula* gum. Anderson (1967) reported a value of 4.3 for pH of Acacia Senegal gum. Karamalla *et al*., (1998) reported 4.66 pH values for *Acacia senegal* and 4.2 for *Acacia seyal* gum.

2.1.5.9. Colour

The colours of gums vary from water- white (colourless) through shades of yellow to black. The best grades of gum are almost colourless with slight traces of yellow; some possess a pinkish colour (Siddig, 2003). On the other hand dark or even black gums some times occur e.g. mesquite gum. There are also the pale rose pink, darker pink and yellowish gums. The pink colour is probably due to the presence of different quantities of tannin materials (Omer, 2004).

2.1.5.10. Equivalent weight and uronic acid anhydride (%)

Uronic acids are widely distributed in animal and plant tissues and constitute a major component of many natural polysaccharides. Various methods have been used for the determination of uronic acids, these Include

A-Colorimetric Techniques:

Uronic acid on heating, are convertd into a furfural type chromogen (5 fotmyl furonic acid; Bowness, 1958). Reaction of carbazole (Diche, 1947; Bitter and Muir, 1962), ornithol (Mejbaum, 1934), anthrone (Helbert and Brown,1961), and meta-hydroxy biphenyl (Blumenkrantz and Asobe-Hansen, 1973) with uronic acid chromogen produces a coloured (Schiffs base) adduct which can be conveniently quantified using uronic acid standards at the appropriate wavelength.

B-Decarboxylation methods:

Decarboxylation of polysaccharides containing uronic acids can occur on heating (100 C) in 12% hydrochloric acid for 4 to 8 houre (Burkart *et al*., 1934) or in 55% hydroiodic acid for two and a half hours (Anderson *et al*., 1963). The carbon dioxide evolved is:

- Absorbed in a standard alkali and back titrated against standard acid and the weight of carbon dioxide absorbed relative to the weight of sample used is equivalent to the uronic acid content.
- Or Collected in a special glass cell (Anderson and Herbich, 1963) and its infra-red (IR) absorption at 2350 nm is recorded Standards of sodium carbonate of accurately known concentrations are treated similarly , their (IR) absorbance noted. A calibration curve is used to determine the uronic acid content as a proportion of the weight of the sample.

C-Acid –alkali analysis:

The equivalent weights of polysaccharides containing uronic acid residues can be determined by titrating a solution containing a known weight of the polysaccharide (in the acid form) against standard alkali.

Karamalla *et al.*, (1998), assessed the potentials of new parameters such as equivalent weight and total uronic acid content as additional qualifying indices. They found that the mean values for gum of *Acacia senegal* for the equivalent weight was 1436 and for uronic acid was 13.71%.

2.1.5.11. Cationic composition (ppm)

(Omar, 2013) carried out study on *Acacia senegal var senegal* cationic composition and the results are reported in Table 2.3.

Table 2.3: *Acacia senegal var. senegal* cationic composition (Omer, 2013)

Sample number	Ca Ppm	Mg Ppm	Na ppm	K ppm
1	12500	1400	2106	4833
2	7371	2841	1860	10916
3	7820	2500	1666	9666
4	3782	3122	166	11500
5	7250	3313	227	5833
6	7500	742	878	6583
7	4500	281	250	8750
8	5512	3654	621	6666
9	6730	3654	60	7416
10	5000	2500	90	8416
Mean	6797	1009	792.4	8057.9
S.D.	2446.1	1895.1	1535.9	1885.3
C.V.	3598	187.8	193.8	23.40

C.D, coefficient of variation.

S.D, standard deviation.

2.1.6. Applications of plant gums

The solubility and viscosity of a gum are the most fundamental properties, which make it unique among polysaccharides, the majority of gums dissolve in water at different concentrations, and such properties are exploited in many applications.

2.1.6.1. Applications in the food industry

Gums, for their high viscosity in solutions and inability to crystallize are, particularly, suited to serve in foodstuff such as: thickeners for beverages, stabilizers for oil and water emulsions and as wider application where function is to prevent agglomeration and setting of minute particles. They are also used to incorporate flavors in confectionery such as

pastilles and gum drops, and the preparation of lozenges. The role of gum Arabic in confectionary products is, usually, either to prevent crystallization of sugar or to act as an emulsifier (Glicksman *et al.,* 1973).

2.1.6.2. Pharmaceutical and cosmetic applications

Gums are used as a suspending and emulsifying or binding agents in pharmaceutical industrys. It has been used in tablet manufacturing, where it functions as a binding agent or as a coating prior to sugar coating, sometimes in combination with other gums (Voget, 1995).

2.1.6.3. Paints and coatings composition application

The hydrophilic colloids and modified cellulose find application in paint industry because of their stabilizing effect on paint emulsions, waxes and numerous others products. Gamble and Grady (1938) treated pigments with water soluble hydrocolloids such as gum arabic to add controllable chemotropic properties to paints. The gum also finds application in coating composition. Horne and Sanko, (1953) developed non8 glare coating based on a water soluble dye dissolved in gum arabic solution.

2.1.6.4. Other industrial uses

Due to their adhesive properties gums have been used in the manufacturing of adhesives for postage stamps and also in the formulations of paints and inks. Gums may serve as a source of monosaccharide, as e.g. mesquite gum (family *Prosopis*) serve as a source of L-arabinose (51%) because of its easier hydrolysis, and availability of the gum in large quantities. Mesquite gum can be dialyzed by addition of ethanol (White, 1947 ; Hudson, 1951), or alternatively, isolated by crystallization from methanol after removal of acidic oligosaccharides on ion exchange resin or precipitated by barium salts. Gums are widely used in textile industries to impart luster to certain materials (silk), as thickeners for colors and mordant in calico printing (Omer, 2004).

2.2. *Acacia senegal*

Acacia Senegal Tree

2.2.1.Botanical Classification

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae*
Genus	*Acacia Mill*
Species	*Acacia sengal (L.) Willd.*

Synonyms

Acacia verek Guill.& perrott, *Acacia rupestris* Stokes, *Acacia trispinosa* Stokes, *Mimosa senegal L.*, Gum-arabic , (FAO, 1995).

2.2.2. General description

Shrub to small tree 2-12 m. Bark yellow to light brown or grey, rough, fissuring or flaking. Yong branchlets grey, yellow or brown, pubescent to glabrous, with horizontal slit lenticles. Stipules not spinescent. Prickles at nodes, in threes two lateral pointing upward or forward and one central pointing downward or backward, falcate, dark brown with a grey base, 4-7

mm. Rachis 2-6 paired, 0.5-3 cm long. Leaflets 8-18 paired 1-6 x 0.5-2 mm, linear to ellipticoblong, ciliclate on margins, glabrous or pubescent, a pex obtuse to subacute. Inflorescence cylindrical spike, 2-10cm long, on peduncles 0.7-2 cm. long, pubescent or glabrous. Flowers white or cream, sessile. Calyx pubescent, 2 x 0.7 mm, 5-6 lobed creamy or pinkish. Corolla glabrous, cream. 0.3 x 2.5 mm, stamens glandular, 4-7 mm long. Ovary glabrous 0.7 mm long; style 4.5mm long; stipe 0.2 mm long. Pods pale brown to straw-color, membranous, dehiscent, pubescent, flat, straight, oblong, a pex rounded to acuminate, 3-14 cm long, 1-3.3 cm wide. Seeds orbicular 8-12 mm diam; yellow or pale brown, compressed; areole central, crescent-shaped, 1.5-6 x 2.5-5 mm; funicle 7.5 mm long. Seed lie vertically on pods. Flowering November-February; Fruiting-April (Elamin, 1990).

2.2.3. Sudan gum belt

Central Sudan, a continuous belt from east to west, on westem sand plains of Kordofan and Darfur; pure or mixed with A, *mellifera*. Widespread in tropical Africa.

2.2.4. Habitat

On sandy and clay plains of savanna grasslands (Elamin, 1973).

2.3. Preparation of glucuronic acid

Until recently there was only one general method for preparation of glucouronic acid, this was the method used by Thomas and Marray (1928).

- Glucuronic acid can be prepared by dissolving commercial gum arabic in about 0.1N hydrochloric acid, ethanol was added with stirring and the precipitate was allowed to settle and then filtered out. The last step repeated four times to give a very pure product. The purified gum was redissolved in water and electrodialyzed for 50 hours. at which time a 1% solution showed a pH of 2.7. The final glucuronic acid kept in solution, since drying with ethanol or distilling under reduced pressure yielded an insoluble product.

- Moorjani and Narwani (1948) prepared glucuronic acid by electrodialysis through cellophane, drying in a water bath, powdering and sieving through a fine mesh screen. They reported that drying glucuronic acid at 110 ˚**C** yielded an insoluble material.

- Recentaly Schleif *et al*., (1951) prepared glucuronic acid by an ion exchange method and, successfully, dried it by spray-drying at 205 ˚**C** to yield a fluffy, white, finely subdivided powder. A similar ion exchange method was used by Wood (1954) to prepared

glucuronic acid. This procedure was also used by Swintosky *et al*., for the preparation of glucuronic acid and several other polysaccharide acids.

2.4. Preparation of glucuronates

Several procedures have been reported for preparing glucuronates. According to Glicksman and Ralph (1959) sodium glucuronates can be prepared as follows:

- Schleif *et al*., (1951) prepared sodium glucuronate using ion exchange methods. Glucuronic acid prepared by ion exchanging was used to prepare spray-dride glucuronates of potassium, calcium, magnesium, zinc, iron and aluminum. Wood (1954) also prepared glucuronates of iron, copper and silver by ion exchange procedure.
- Amore simplified one-step ion exchange process for preparing glucuronates was patented by Adams (1977), Glucuronic acid was passed through a cation – exchange resin in the salt form, high purity glucuronates were prepared.
- Briggs (1934) used direct neutralization of glucouronic acid solution with sodium hydroxide followed by evaporation to dryness under reduced pressure at 70. Similar methods have been used for preparing calcium glucuronate and other salts.

2.5. Ion exchange chromatography

Ion exchange chromatography is an exchange of ions between two electrolytes or between an electrolyte solution and a complex. In most cases the term is used to denote the processes of purification, separation, and decontamination of aqueous and other ion-containing solutions with solid polymeric or mineralic 'ion exchangers'.

Typical ion exchangers are ion exchange resins (functionalized porous or gel polymer), zeolites, montmorillonite, clay, and soil humus. Ion exchangers are either cation exchangers that exchange positively charged ions (cations) or anion exchangers that exchange negatively charged ions (anions). There are also amphotric exchangers that are able to exchange both cations and anions simultaneously. Howerver, the simultaneous exchange of cations and anions can be more efficiently performed in mixed beeds that contain a mixture of anion and cation exchange resins, or passing the treated solution through several different ion exchange materials (Dorgner, 1992).

2.6. Emulsification Properties of *Acacia senegal* Gum, Glucuronic acid and Glucuronates

2.6.1. Definition of emulsion

An emulsion is a dispersed system that consists of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small droplets in the other called continuous phase stabilized by presense of emulsifying agent. Emulsions are, thermodynamically, unstable systems and have a tendency to break down over time. The breakdown of an emulsion may manifest itself through different, physicochemical, mechanisms such as gravitational separation, coalescence, flocculation, Ostwald ripening and phase inversion. Therefore, the production of high quality food emulsions that can remain, kinetically, stable for a certain period of time is necessary (Sabah El-Kheir *et al*., 2008).

2.6.2. Classification of emulsion

Emulsions can be classified, according to the relative spatial distribution of the different phases, into three types as follows:

- Oil in water(O/W) emulsion, which is an emulsion consisting of oil droplets dispersed in an aqueous phase such as dips, cream, beverages, milk, dressings and ice-cream.
- Water in oil(W/O) emulsion, that consists of water droplets dispersed in an oil phase such as margarine, butter and some spreads.
- Also it is possible to create various types of multiple emulsions, such as water in oil in water(W/O/W), oil in water in oil(O/W/O) and 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 micro fluidizer or an ultrasonic homogenizer (McClements, 2007).

2.6.3. Emulsion formation

Emulsions can be prepared by, easily, blending the immiscible liquids in the presence of a, very important, third component, which is a surfactant. When a surfactant is used in emulsification, it is, widely, known as an emulsifier. The degree of agitation required depends on the properties of the liquids, which will influence, at least, the resulting

viscosity. This in turn, will somehow dictate the degree of intensity of mixing enabling the breaking of the oil into droplets. Agitation may come in the form of a simple kitchen blender, homogenizer or ultrasonic.

In most manufacturing industries, emulsification is achieved using the homogenizer. For straightforward preparation, Bancroft rule can be used in determining the type of emulsion: o/w or w/o. The rule proposes that the phase into which the emulsifier is dissolved will become the continuous one. Another useful guide is the hydrophile-lipophile balance (HLB). This is a value ascribed to a surfactant based upon the chemical composition and its hydrophilicity. Emulsion behavior and stability are affected by the properties of the adsorbed layers that stabilize the oil-water interfaces. In this case the adsorbed layers consist of molecules of surfactants. Determination of the surface tension may give some ideas on the possibility of emulsification but insufficient to understand stability, phase separation or the rheological behavior (Mullins and Sheu, 1995).

2.6.4. Emulsifying agents

Emulsifying agents (EAs) are very important ingredients having an essential role to ensure emulsification and stability in the formation of emulsions in aqueous solutions (Krog and Sparso, 2004; McClements, 2005). EAs are amphiphilic molecules including both hydrophilic and lipophilic parts (Zhang, 2011). EAs are surface-active compounds being absorbed on the surface of droplets and ensuring kinetic stability in a certain period by being added into emulsion before or after homogenization (McClements, 1999; McClements *et al*., 2007). Components like thickening agents and stabilizing agents used in the formation of emulsions are different and shouldn't be confused with the term of emulsifying agent. Thickening agents typically increase the viscosity in the continuous phase of emulsion and limit the movements of droplets and develop emulsion stability in this way. Stabilizing agents are used to increase the stability provided by both emulsifying agents and thickening agents (McClements, 1999).

The principal effects of emulsifying agents could be listed as follows:

- The decrease of the interface tension between water-oil phase or water-air phase and to create an interface membrane by providing structural or electrostatic interactions between droplets,
- The decrease of the required amount of energy for the degradation of coarse particles and thus ensures the formation of smaller droplets,
- prevent ion of coalescence by creating a protective layer surrounding droplets,

- provision of additional functions like modifying oil crystallization, making interactions with carbohydrate components, forming films and controlling the transport of oxygen or moisture (McClements, 1999; Krog and Sparso, 2004; McClements, 2005; Zhang, 2011)

A number of food components exhibiting the mentioned characteristics (e.g. lecithin, proteins, gums, modified starches, phospholipids, etc.) could be used as EAs. However, these components vary, considerablys in terms of their molecular structure, which influences their ability to form and stabilize emulsions, as well as withstand environmental stress, such as variations in ionic strength, pH and temperature (Zhang, 2011). One of the common problems encountered, by researchers, is to form stable multiple emulsions with food-grade EAs instead of, synthetic, surface-active components and polymers. In order to ensure stability in non-food emulsion products, utilizing sorbitan esters and, synthetic, co-polymers is a common and simple method. In order to develop emulsion applications in food, researchers mainly focus on the usage of food proteins and polysaccharides (Kanouni *et al*., 2002; Benichou *et al*., 2007; Dickinson, 2011).

In order to stabilize primary emulsions in W/O/W food emulsions, in other words to stabilize the droplets in internal phase, there have been several studies where various biopolymers like gum *Acacia* (Vaziri and Warburton, 1994; Su *et al*., 2008), xanthan gum (Evison *et al*., 1995), gelled starch (Iancu *et al*., 2009) have been utilized. Colloidal particles of many polysaccharides such as carrageenan, locust bean gum (Suzuki and Lim, 1994; Benna-Zayani et al., 2008; Perrechil and Cunha, 2012), xanthan gum (Benna-Zayani *et al*., 2008), gum arabic (Lobato-Calleros *et al*., 2008; Xu *et al*., 2011) have been utilized serving as thickening/gelling agents in stabilizing the droplets of secondary, emulsions. i.e. external phase of multiple emulsions. It could be possible to ensure optimum stability by selecting and using, appropriate, proteins and polysaccharides together in, primary and secondary, emulsions to obtain a synergistic effect. Since the convenience of EAs used, the formulation and composition of the emulsion and the emulsification methods are main effective factors on stability issues, by taking all of these points into account, it could be possible to obtain acceptable emulsions protecting their structural stability for a desired time.

2.6.5. Emulsifying properties

Emulsions are chemical mixtures of liquids that are immiscible under ordinary conditions, and which may be separated into two layers on, standing, heating and freezing, by agitation and the addition of other chemicals (Encyclopedia, 1966). The emulsifying agents act as surface – active agents, which when added to an emulsion it would increase its stability by interfacial action, each emulsifying agent depends on its action on different principle to achieve a stable product. Gum arabic is used to stabilize flavor and oil emulsions in dried food mixes (such as soup, cakes,…etc) and the soft drinks industry, where the gum is required to stabilize a concentrated oil emulsion (about 20%) for long periods and also to continue to stabilize following dilution prior to bottling (Islam *et al*., 1997). An emulsifying agent is, usually, a long – chain organic compound that produce chains that are soluble in oil (lipophilic) as well as side chains or groups that are soluble in water (hydrophilic). Thus one portion of each molecule dissolve in the water phase while another portion dissolves in the oil phase and the main chain forms a link or bridge to keep both phases in position and there by emulsification. Gum arabic produces highly stable emulsions making it very useful in the preparation of oil in water food flavour emulsions particularly, for citrus oils (Randall *et al.*, 1988). Some believe that gums are not true emulsifiers. That is, they do not act by means of hydrophilic chemical functionality; they perform as emulsion stabilizers or protectors. Their function 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 be, stabilized. Such stabilization is a protective effect based on thickening properties of the gums. (Randall *et al.,* 1988) studied the effect of heat on the emulsification action, stability of the gum followed by its changes in the GPC profile.

He concluded that heating at 100ºC for 3 hours, results in a decreases in the intensity of the high molecular mass peak with a corresponding increase in the intensity of the lower molecular mass peaks. Continuous heating leads to further loss of the high molecular mass fraction and loss in the emulsifying stability of the gum. Chikamai and Banks, (1993) reported that heating solutions at 100ºC for more than six hours causes significant loss of emulsification properties; where as, heating at 60ºC for 24 hrs has only a minor effect. Dickinson and Sainsby, (1988) studied the surface and emulsifying properties of six *Acacia* gum samples and stated that the relationship between nitrogen content and emulsifying properties of the gum samples, depend not only on their total protein content, but also on the distribution of the protein/peptide between the low and high molecular weight fractions,

and on the molecular accessibility of the protein/peptide for adsorption. Dickinson *et al*.(1991) studied the influence of the nature of the oil phase on emulsifying behavior of gum arabic. They found that gum lowered the surface tension at the n-hexadecane-water interface. It gave also the most stable n-hexadecane – water emulsion with the smallest droplets in two oils (n-hexadecane and orange oil). They also concluded that a high molecular weight fraction (0.87 nitrogen) corresponds to 10% of a natural gum (0.38% nitrogen) gives initially larger droplets but better emulsion stability than the low molecular weight fraction (0.35%). In common with most emulsifiers, the AGP complex has a hydrophilic region (protein) and hydrophilic region (carbohydrate). During the formation of oil in water emulsions the protein (arabinogalactan) protein products in to the water phase. The bulk of gum arabic in the form of free AG can improve stability by increasing viscosity of the water (Islam *et al.,* 1997). The, relatively, low protein content of gum arabic requires high concentration of gum in most emulsification systems (Imerson, 1997).

2.6.6. Applications of emulsion

Emulsions can be used in variety of fields:

2.6.6.1. In Foods

Oil-in-water emulsions are common in food; it can be used in:

- Mayonnaise and Hollandaise sauce – these are oil-in-water emulsions that are stabilized with egg yolk, lecithin or with other types of food additives, such as sodium stearoylate.
- Creams(foam) in espresso – coffee oil in water(brewed coffee), unstable emulsion.
- Homogenized milk–an emulsion of milk fat in water and milk proteins water-in-oil emulsions are less common in food but still exist:
  -Butter – an emulsion of water in butterfat.
- Vinaigrette – an emulsion of vegetable oil in vinegar. If this is prepared using only oil and vinegar (i.e. without an emulsifier), an unstable emulsion results (Mason *et al*., 2006).

2.6.6.2. In Pharmaceutics and medicines

In pharmaceutics, hairstyling, personal hygiene, and cosmetics, emulsions are frequently used. These are usually oil in water emulsions, but which is dispersed and which is continuous depends in many cases on the pharmaceutical formulation. These emulsions may be called creams, ointments, liniments (balms), pastes, films, or liquids, depending mostly on their oil-to-water ratios, other additives, and their intended route of

administration. The first five are topical dosage forms, and may be used on the surface of the skin, transdermally, ophthalmically, rectally, or vaginally. A highly liquid emulsion may also be used orally, or may be injected in some cases. Popular medications occurring in emulsion from include calamine lotion, cod liver oil, polysporin, cortisol cream, canesten, and fleet.

Microemulsions are used to deliver vaccines and kill microbes. Typical emulsions used in these techniques are nanoemulsions of soybean oil, with particles that are 400-600 nm in diameter. The process is not chemical, as with other types of antimicrobial treatments, but mechanical. The smaller the droplet, the greater the surface tension, and thus the greater the force required to merge with other lipids. The oil is emulsified with detergents using a high-shear mixer to stabilize the emulsion when they encounter the lipids to merge with themselves. On a mass scale, this effectively disintegrates the membrane and kills the pathogen. The soybean oil emulsion does not harm normal human cells, or the cells of most other higher organisms, with the exceptions of sperm cells and blood cells, which are vulnerable to nanoemulsions due to the peculiarities of their membrane structures. For this reason, these nanoemulsions are not currently used intravenously. The most effective application of this type of nanoemulsion is for the disinfection of surfaces. Some types of nanoemulsions have been shown to effectively destroy HIV-1 and tuberculosis pathogens on ono-porous surfaces.

2.6.6.3. In Firefighting

Emulsifying agents are effective at extinguishing fires on small, thin-layer spills of flammable liquids (Class B fires). Such agents encapsulate the fuel in a fuel-water emulsion, thereby trapping the flammable vapors in the water phase. This emulsion is achieved by applying an aqueous surfactant solution to the fuel through a high- pressure nozzle. Emulsifiers are not effective at extinguishing large fires involving bulk/deep liquid fuels, because the amount of emulsifier agent needed for extinguishment is a function of the volume of the fuel, whereas other agents such as aqueous film-forming foam (AFFF) need to cover only the surface of the fuel to achieve vapor mitigation (Silvestre *et al*., 1999).

2.7. Turbidity of Fluids

2.7.1. Definition

Turbidity is the measure of the “cloudiness” of water; more precisely, it measures the extent to which light is scattered and absorbed by a suspended sediment, dissolved organic matter, and, to a lesser extent, plankton and other microscopic organisms (Clesceri *et al*., 1994).

From a technical standpoint, turbidity is a relative measurement of scattering as compared to a calibrated standard, usually a formazin suspension (Davies-Colley and Smith 2001). Turbidity is also referred to as the inverse of the “clarity” of water. Light that is not scattered or absorbed by turbidity-causing particles passes through the water. In other words, increased turbidity reduces the distance that light can penetrate into the water column.

2.7.2. Causes of turbidity

Turbidity is caused by suspended matter or impurities that interfere with the clarity of the solution. These impurities may include clay, silt, finely divided inorganic and organic matter, soluble colored organic compounds, and plankton and other microscopic organisms (EPA, 1999).

2.7.3. Theoretical backgrounds

A directed beam of light remains, relatively, undisturbed when transmitted through, absolutely, pure solution. Molecules in a pure fluid will scatter light to a certain degree. Therefore, no solution will have zero turbidity. In samples containing suspended solids, the manner in which the sample interferes with light transmittance is related to the size, shape and composition of the particles in the solution and to the wavelength (color) of the incident light (Sadar, 1998).

A minute particle interacts with incident light by absorbing the light energy and then, as if a point light source itself, re-radiating the light energy in all directions. This omnidirectional re-radiation constitutes the "scattering" of the incident light. The spatial distribution of scattered light depends on the ratio of particle size to wavelength of incident light. Particles much smaller than the wavelength of incident light exhibit a fairly symmetrical scattering distribution with approximately equal amounts of light scattered both forward and backward (Sadar, 1998).

As particle sizes increase in relation to wavelength, light scattered from different points of the sample particle create interference pattems that are additive in the forward direction. This constructive interference results in forward-scattered light of a higher intensity than light scattered in other directions. In addition, smaller particles scatter shorter (blue) wavelengths more intensely while having little effect on longer (red) wavelengths. Conversely, larger particles scatter long wavelengths more readily than they scatter short wavelengths of light (Sadar, 1998).

Particle shape and refractive index also affect scattered light distribution and intensity. Spherical particles exhibit a larger forward-to-back scatter ratio than coiled or rod-shaped particles. The refractive index of a particle is a measure of how it redirects light passing through it from another medium such as the suspending fluid. The particles refractive index must be different than the refractive index of the sample fluid in order for scattering to occur. As the difference between the refractive indices of suspended particle and suspending fluid increases, scattering becomes more intense (Sadar, 1998).

The color of suspended solids and sample fluid are significant in scattered-light detection. A colored substance absorbs light energy in certain bands of the visible spectrum, changing the character of both transmitted light and scattered light and preventing a certain portion of the scattered light from reaching the detection system (Sadar, 1998).

Light scattering intensifies as particle concentration increases. But as scattered light strikes more particles, multiple scattering occurs and absorption of light increases. When particulate concentration exceeds a certain point, detectable levels of both scattered and transmitted light drop rapidly, marking the upper limit of measurable turbidity. Decreasing the path length of light through the sample reduces the number of particles between the light source and the light detector and extends the upper limit of turbidity measurement (Sadar, 1998).

2.7.4. Turbidity measurement

Turbidity can be measured using either an electronic turbidity meter or a turbidity tube. Turbidity is usually measured in nephelometric turbidity units (NTU) or Jackson turbidity units (JTLJ), depending on the method used for measurement. The two units are roughly equal.

2.7.5. Turbidity meter

There are many different types of electronic turbidity meters available. In this study, 2100N Turbidity meter is used Fig 2.3. The 2100N Laboratory Turbidimeter operate on the principle that the amount of light scattered from a sample is proportional to the quantity of particulate material in that sample. Light from a tungsten halogen lamp, operating at a nominal color temperature of 270 ^{o}K, is collected by a set of three polycarbonate lenses. The polycarbonate is able to withstand the temperature extremes from the lamp. The lenses are designed to gather as much light as possible and image the filament of the lamp to the sample cell. A blue infrared (IR) filter in the optical path causes the detector response to

peak at a wavelength between 400 and 600 nanometers, in compliance with EPA guidelines. 2100N turbidimeter use the forward-scatter detector to linearize instrument response at high turbidities.

The stray ligh specification of the 2100N turbidimeter less than 0.01 NTU for the laboratory models. Calibration is automatic, quick and simple (Sadar, 1998).

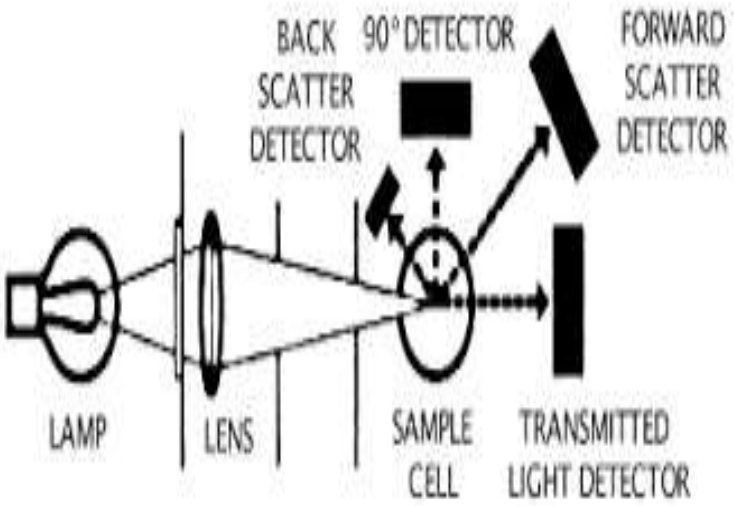

Fig 2.3: Turbidity meter diagram

2.8. Spectrophotometry and Spectroscopy

2.8.1. Absorption spectroscopy

It is the measurement of selective absorption by atoms, molecules, or ions of electromagnetic radiations at definite and narrow wave length range, approximating monochromatic light. Absorption spectrophotometry encompasses the following wave length regions: ultraviolet (185 to 380nm), visible (380 to 780 nm), near infra-red (780 to3000 nm) and far infra-red (500 to 40000 nm).

UV-visible spectroscopy is the measurement of the absorbance of light at a specific wavelength in a sample. This is used to identify the presence and concentration of molecular entities within the sample. The Beer-Lambert law is used to relate the absorption of light to the properties of the sample through which the light is travelling through. The Beer-Lambert law states that:

$$A = \varepsilon IC$$

A is the absorbance

ε is the molar absorption coefficient (l $mol^{-1}cm^{-1}$)

C is the concentration (mol l^{-1}), **l** is the path length (cm)

This law shows that absorbance is linear to concentration but this is only true for low concentrations. For absorbance levels above 3 the concentration starts to move away from the linear relationship.

Transmittance is the proportion of the light which passes through the sample:

$$T = \frac{\mathrm{It}}{Io}$$

Where:

I_o is the incident light

I_t is the transmitted light

2.8.2. Instrument description

The 6305 spectrophotometer is suited to a wide range of applications in education, quality control, environmental and clinical analysis. This model is a UV/visible spectrophotometer covering a wavelength range from 198nm to 1000nm, with measurement modes for absorbance, % transmittance and concentration. It has full interfacing capability for Analogue output and serial interfacing (RS232). The optical system is independently housed and isolated with lenses to give maximum protection from environmental contamination. Combined with a mechanically rigid structure, this model provides a system with fast warm-up, low drift and high reliability.

3. Hypothesis

Potassium glucuronate give a more stable emulsion than the parent gum from *Acacia senegal* var. *senegal*.

4. Objectives

1-To determine the physicochemical properties of *Acacia senegal* gum.

2-To Prepare and characterize glucuronic acid and potassium glucuronate.

3-To Study the stability of emulsions using *Acacia gum*, glucuronic acid and potassium glucuronate using Nephlo metry and UV/VIS spectroscopy.

Chapter Two

Materials and Methods

Chapter two

Materials and Methods

2.1. Materials

A sample of *Acacia senegal* was used . The Sample was collected from Al gadaref, season 2015.

2.2. Methods

2.2.1. Sample preparation

The gum sample used in this work was cleaned by hand to be sure it was free from sand, dust and bark impurities, it was ground using pestle and mortar, then kept in labeled (polyethylene) bags.

2.2.2. Preparation of glucuronic acid and potassium glucuronate

Glucuronic acid was prepared using ion exchange chromatography method. A glass column packed with an amberlite resin IR 120 H+ (a strong cation exchange resin) was used. After the sample was completely, dissolved, the solution was left until it became free from bubbles and insoluble matter before use. The solution was slowly passed through the column in order to replace its cations by the hydrogen bonded to the resin; the collected eluent was arabic acid (Glicksman and Schachat, 1959). Solution of potassium glucuronate was prepared by titrating the arabic acid with potassium hydroxide solution (0.1M). The solution prepared by the above procedure was dried using a Freeze Dryer (Model Edwards, England) A fluffy, white, fine powder was obtained.(Encyclopedia, 1966).

2.2.3. Determination of moisture content (%)

According to FAO (1990) the moisture content of gum arabic, glucuronic acid and potassium glucuronate were determined as follows:

Samples were accurately weighed, heated in an oven (Memmert, Modell 100-800) at 105 ˚C for 5 hours . The dry specimens were allowed to cool in a desiccators, and the loss of weight was calculated as percentage from the initial weight using the following relation.

$$\text{Moisture \%} = \frac{\text{weight of water evaporated in grams x100}}{\text{weight of sample in grams}}$$

2.2.4. Determination of total ash content (%)

Total ash in gum arabic, glucuronic acid and potassium glucuronate were estimated according to AOAC method (1984) as follows:

Crucible were heated in an oven (at 105 ˚C) for 30 minutes cooled in a desiccator and then weighed (W_1). About two grams of sample were placed in the crucible and accurately weighed (W_2), then ignited at 550^{o}C in muffle furnace (Nabertherm, Model B150) for 2 hours, cooled in a desiccater and weighed (W_3). Total ash% was calculated as follows:

$$\text{Total ash content } \% = \frac{W3-W2}{W2-W1} \times 100$$

Where

W_1: Weight of the empty crucible

W_2: Weight of crucible +sample

W_3: Weight of crucible +sample after ashing

2.2.5. Determination of nitrogen and protein content

Nitrogen was determined using a semi-micro khjeldahal method as described by AOAC (1984). Accurately weighed 0.2 gram of gum samples were taken in triplicates in khjeldahal digestion flasks then a khjeldahal tablet (Copper sulphate-potassium sulphate) along with 3.5 mls of concentrated nitrogen free sulphuric acid were added to each flask .The flasks and contents were then heated over an electric heater until the solution attained a clear blue color and the walls of the flask were free from carbonized materials. The contents of the flask were then transferred to a steam distillation unit (BÜCHI, B.323.SWITZERLAND), and 15 mls of 40% sodium hydroxide solution were added, and distillation was carried out. The distillate was then collected in 10 mls of 2% boric acid solution with three drops of methyl red indicator, and titrated against 0.01 N HCl. The same procedure was carried out for a blank (distilled water).

$$\text{N\%} = \frac{(\text{M1} - \text{M2})\text{x N x } 14.01 \text{ x } 100}{\text{S x } 1000}$$

Where:

M_1: mls of HCl that neutralized the sample distillate

M_2: mls of HCl that neutralized the blank distillate

N : Normality of HCl titrate (0.01)

S : Sample weight (0.2g)

The reactions involved in these steps are as follow:

Sample $+H_2SO_4$ (conc.) + Catalyst +heat $\rightarrow (NH4)_2SO_4$

$(NH4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

$NH_3 + H_3BO_3 \rightarrow NH_4 + H_2BO_3$

The protein content was determined by multiplying % nitrogen percent by a NCF of 6.6 (Anderson, 1986)

2.2.6.Determination of specific optical rotation

The specific optical rotation was determined according to FAO (1991). A 1.0% solution (on dry weight basis) was measured at room temperature, using an optical activity Polarimeter type NewClssic MF automatic polarimeter (ENGLAND) fitted with a sodium lamp with a cell path length of 20 cm. The solution was passed through a No.42 filter paper before carrying out measurements at room temperature. Triplicate readings were taken and averaged. The specific optical rotation for gum solution was calculated according to the relationship:

Specific optical rotation = $\alpha \times 100 / C \times L$

Where:

α= Observed angular rotation

L= Length of the polar meter tube in decimeters

C= Concentration of the solution expressed as number of grams substance in 100 cm^3 of solution

2.2.7.Determination of viscosity

Viscosity was measured using U-tube viscometer(type BS/ IP/ U, Serial No. 2948) with the flow time for 1% aqueous solution of formulation at 25°C, The solvent used was NaCl of 0.5 concentration. The relative viscosity(η_r) was then calculated using the following equation:

$$\boldsymbol{\eta_r = \frac{T - To}{To}}$$

Where:

T = flow time of formulation solution expressed in seconds.

T_o = flow time of solvent (NaCl) expressed in seconds.

The reduced viscosity (η_{rd}) was determined for different concentrations of gum solution 0.2, 0.4, 0.6, 0.8 and 1% and was then calculated from the following equation:

$\eta_{rd} = \eta_r / C$

Where:

η_{rd} = reduced viscosity

η_r = relative viscosity

C = concentration of formulation solution

The intrinsic viscosity (η) was obtained by extrapolation of reduced viscosity against concentrations back to zero concentration. The interception on Y – axis gives (η).

2.2.8.pH measurement

The pH value was determined for 1% aqueous solution at room temperature, using pH meter (JENWAY. Type 3505).

2.2.9. Determination of the total glucuronic acid

The method used for the determination of uronic acid in gum arabic in this work was the acid-alkali analysis. According to Encyclopedia (1966) as follows:

A cation exchange column packed with Amoerlite (IR-120+) resin was thoroughly washed with 2.0 M Sulphuric acid , followed by distilled water until the column was sulphate free. Gum Arabic sample (50 ml of 2 to 3%w/v) was slowly passed down the column. The eluent and washing (~ 300 ml) was collected and titrated against standard Sodium hydroxide (0.1M) Solution using phenolphthalein as an indictor. The equivalent weight was calculated as follows:

$$\text{Acid equivalent weight} = \frac{\text{weight of sample x 100}}{volume\ of\ titre\ x\ molarity\ of\ alkali}$$

Uronic acid percentage was determined according to Elamin (2001) by multiplying the molecular weight of uronic acid (194) by 100 and dividing by apparent equivalent weight of the sample as follow:

Total Uronic acid = 194 x 100/ Equivalent weigh

194 = Molar mass of uronic acid anhydride.

2.2.10.Determination of colour

Colour was determined according to FAO (1991). A concentrated solution of gum arabic 25%(w/w) was prepared. The colour determined by using a colorimeter (Lovibond, Model F).

2.2.11.Determination of molecular weight

The molecular weight was calculated using Mark-Houwink equation(Mark, 1938., Houwink, 1940).

$(\eta) = K \times Mw^{a}$

Where:

(η) = Intrinsic viscosity

Mw = Molecular weight

K and a = Mark -Houwink constant

Based on (Anderson and Rahman, 1967), the values of K and a, were determined for *Acacia senegal* gum as follow:

$K = 1.3 \times 10^{-2}$

a = 0.54

2.2.12.Determination of cationic composition

Dry ashing method was used in sample preparation, 1g of the gum sample was placed in a furnace and heated to 550°C , maintain the temperature for four hours , and cool the sample. Then added 10 ml of hydrochloric acid 3N to the sample, watch glass was used to covered the sample and heated gently for 10 min, then cooled and filtered into 100 ml volumetric flask and dilute with a distill water to the mark. Flame photo meter (JENWAY, Model pFp7) was used to determine the element.

2.2.13.Measurement of stability of emulsions prepared from different emulsifiers and different types of oils

Two types of refined oil (groundnut, sunflower) and 25% w/w aqueous *Acacia senegal* gum**,** glucuronic acid and potassium glucuronate solution (by hydrating them over night) were used

to prepare emulsions by the following concentrations: refined oil 6.5%; *Acacia senegal*, glucuronic acid and potassium glucuronate 20% and deionized water 75%(Buffo, 2001).

The first reading was taken at zero time using a spectrophotometer (6505, uv/vis) at 520nm and turbidity meter (HACH,2100N), the following readings were taken after 24, 48, 72, 96, 120 and 144 hours. Emulsion stability was calculated as follows:

$$\text{Emulsion stability} = \frac{\text{First reading at zero time}}{\text{Reading at x } time}$$

If stability equals one that mean the emulsion is more stable, and if it is not, the emulsion is less stable.

Chapter Three

Results and Discussion

Chapter Three

Results and Discussion

3.1.Physicochemical Properties of *Acacia senegal* gum, glucuronic acid and potassium glucuronate

A number of physicochemical properties were used to characterize the studied sample of *Acacia senegal* gum, glucuronic acid and potassium glucuronate. Table 3.1 shows Physico-chemical properties of *A. senegal var. senegal* gum, glucuronic acid and Potassium glucuronate. The moisture content of *A. senegal* was found to be 8.4%, while glucuronic acid and potassium glucuronate have the moisture contents of 6.8% and 7.1% respectively. Total ash of the gum was found to be 3.2%, while glucuronic acid and potassium glucuronate have total ash contents of 0.3% and 7.7% respectively.

Table 3.1: Physico-chemical properties of *A. senegal var. senegal* gum, glucuronic acid and potassium glucuronate

Property	*A senegal* gum	Glucuronic acid	Potassium glucuronate
Moisture (%)	8.4	6.8	7.1
Total ash (%)	3.2	0.3	7.7
Nitrogen (%)	0.227	ND	ND
Protein (%)	1.5	1.5	1.5
Acid equivalent weight	1700.7	1700.7	ND
Total glucuronic acid (%)	11.4	11.4	ND
PH	4.6	2.7	6.8
Intrinsic viscosity (ml/g)	15.3	10.8	12.9
Molecular weight	4.85×10^5	ND	ND
Specific optical Rotation	-27.5	-20	-25

ND = not determined

Total ash% content indicates the presence of inorganic elements, Anderson and Dea (1968). FAO (1991) specifies that the total ash content as a purity test for gum arabic should not exceed 4%. The results obtained agree with the limit of the specifications. The pH of *Acacia senegal* gum aqueous solution was found to be slightly acidic 4.6 but glucuronic acid and potassium glucuronate have the pH values of 2.7 and 6.8 respectively.

A. senegal has optical rotation value of -27.5˚, while the glucuronic acid and potassium glucuronate have the specific optical rotation of -20˚ and -25˚. FAO (1991) specifies that the aqueous solutions of *Acacia senegal* gum is levorotatory. The result agree with the specified limits of the FAO (1991). Results indicate that the nitrogen content was 0.227% (1.5% protein). In this study, the nitrogen content 0.227% agreed with the range (0.225%-0.425%) reported by Karmalla *et al*.,(1998). It disagree with the value (0.37%) obtained by Anderson (1986) and (0.33%-0.36%) obtained by Osman (1998). The protein content was 1.5% agreed with the range (1.5-3%) reported by Anderson *et al*.,(1985). But disagree with the mean value of 2.31% reported by Omer (2013).

The acid equivalent weight of 1700.7 obtained was within the range (1136-1875) reported by Karmalla *et al*.,(1998). The total uronic acid was found 11.4%. result obtained was within (10.34%-23.32%) reported by Karamalla *et al*.,(1998). But lower when compared to the value of 17% obtained by Jurasek (1993).

Results obtained the color varies from water-white (colour less). while molecular weight of 4.85×10^{5}, Application of the Mark-Houwink equation indicated that the average molecular weight of *Acacia senegal* gum was 4.6×10^{5}(Alamin and Norbest., 1985). the value reported here was higher than the previous value.

Acacia senegal gum has intrinsic viscosity of 15.3ml g^{-1}. while glucuronic acid and potassium glucuronate have intrinsic viscosities of 10.8 and 12.9 respectively. The intrinsic viscosity of glucuronic acid solution decreased with the addition of metal ions and it further by decreased with increasing concentration of NaCl (Schleif, 1951). The results are in good agreement with these reported by the previous, The intrinsic viscosity of the potassium glucuronate was higher when compared to the viscosity of glucuronic acid and this may be due to the polar groups in gum which are sufficiently neutralized with counter ions (salts ion) so the repelling effect increases in diluted NaCl solutions hence, reducing the electrostatic repulsion, making the network more compact and

formed. FAO (1991) includes in its definition of gum arabic that it consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts.

Table 3.2: Cationic composition of *Acacia senegal* gum sample (ppm)

Element	K	Mg	Ca	Na
Concentration/ppm	1952.490	1564	916.910	8.520,

Table 3.2 shows that calcium, magnesium, potassium and sodium are the major elements in *A. senegal var. senegal* sample. Results show that k the major element present in sample (1952.490 ppm) and Na is the less element present in the sample (8.520 ppm). Results agreement with the results of Omar, 2013.

3.2.Emulsification Properties of *Acacia senegal* gum, Glucuronic acid and Potassium Glucuronate

The emulsions and colloidal systems are thermodynamically unstable systems and tend to destabilize due to an excess surface free energy, where stability implies no tendency towards structural changes (Pittia, Gambib, and Lerici, 1997). Consequently, the droplets of the inner phase tend to cluster together spontaneously, forming small or large flocs (flocculation), to coalesce giving larger spherical droplets and cream, leading to a layer of the lower density phase on top of the emulsion (McClements, 1999). Long-term stability of a dispersed system implies that the rate and extent of changes in emulsion structure are adequately low in real time. The long-term stability of emulsion is normally extended by adding a variety of stabilizers (e.g. thickening agents, surfactants, etc) to retard the droplet aggregation or flocculation (Tesch and Schubert, 2002). The emulsion stability is influenced by the balance between attractive (Van der Waals and osmotic) and repulsive (electrostatic, steric and hydration) forces between the emulsion droplets (McClements, 1999). The stability of emulsion is very important for various industrial processes. It is very difficult to maintain the stability of an emulsion (Bibitte, 1992). Many attempts have been made to make emulsion stable, such as, by using emulsifier, salts and polymers (Marquez, 2010). The addition of protein/ polysaccharides may increase the viscosity of aqueous phase and so increase the viscosity and stability of W/O emulsions (Snoeren *et al*., 1976). Further by using different devices for emulsification, the effect of salt on the particle size distribution,

effect of water contents on sedimentation and coalescence process have been investigated with respect of coalescence time.

- **Turbidity measurement**

The measurement of turbidity is a key test of emulsion quality. Emulsion can contain suspended solid matter consisting of particles of many different sizes, very small particles will settle only very slowly or not at all if the sample is regularly agitated or the particles are colloidal. These small solid particles cause the liquid to appear turbid.

The emulsion stability (stability at (0) time over stability at(x) time vs time), as affected by different oil types using turbidity measurement are shown in Fig: 3.1, 3.2, and 3.3. In all Figures *Acacia senegal* gum and potassium glucuronate had good emulsification properties for oil-water emulsion, glucuronic acid is less valid than *A. senegal* gum due to its poor emulsification performance.

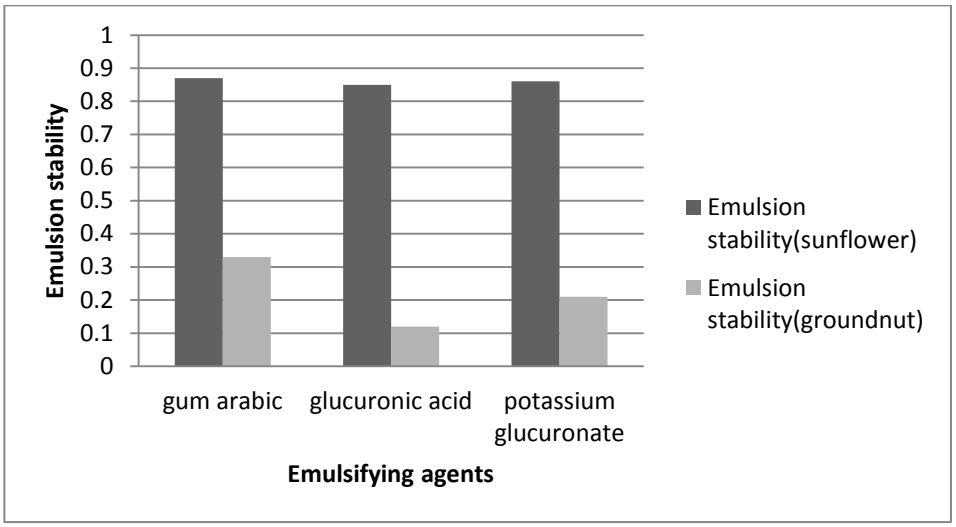

Fig 3.1: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate as affected by different oil types after one day using turbidity measurement

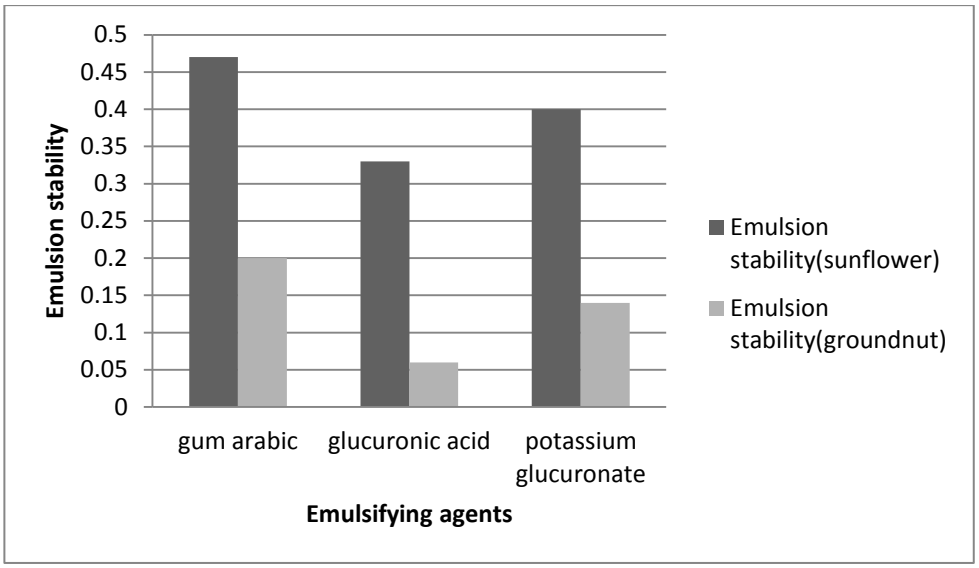

Fig 3.2: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate as affected by different oil types after three days using turbidity measurement

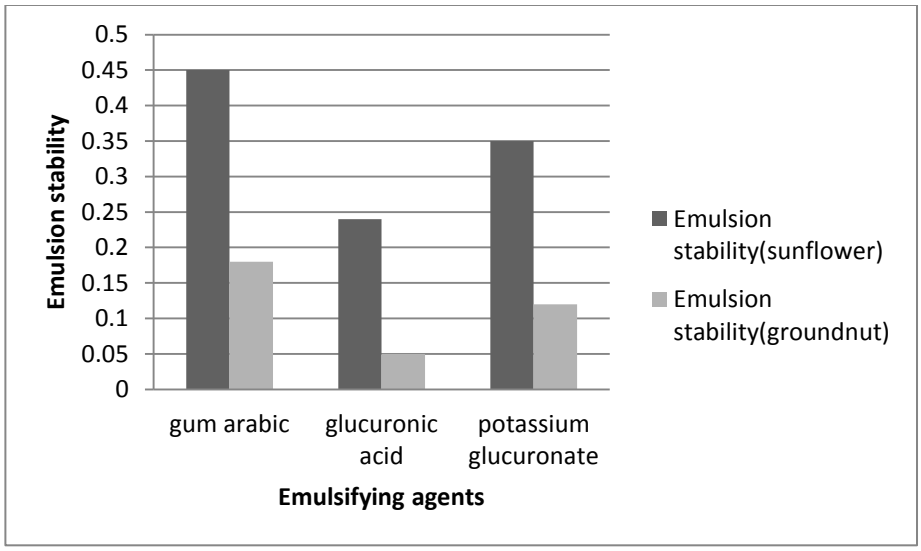

Fig 3.3: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate as affected by different oil types after seven days using turbidity measurement

- **Absorption measurement**

UV-Visible spectroscopy was used to monitor the solution turbidity as an evidence of the dispersion of the emulsion or study of emulsion stability.

The emulsion stability (stability at (0) time over stability at(x) time vs time) as affected by different oil type using UV measurement are shown in Fig: 3.4, 3.5, 3.6 and 3.7. Results obtained the *Acacia senegal* and potassium glucuronate have a good emulsification properties if compared to the glucuronic acid.

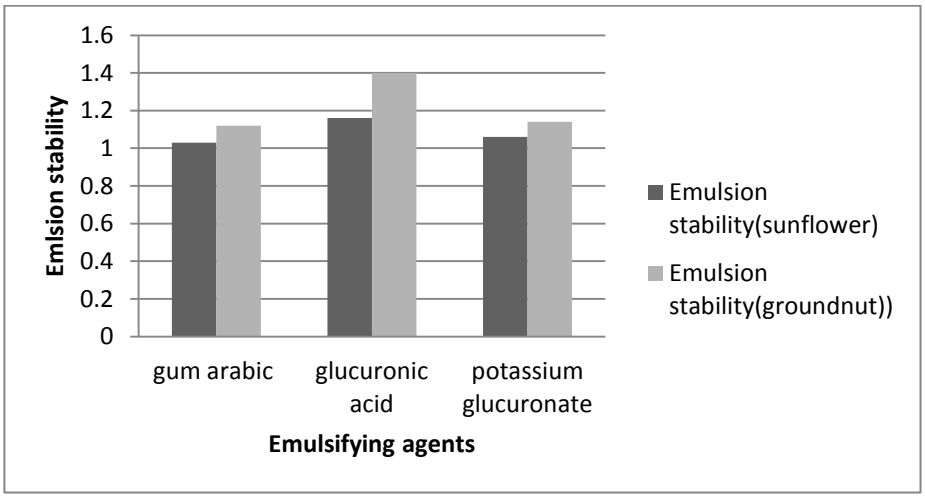

Fig 3.4: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate emulsions as affected by different oil types after one day using UV measurement

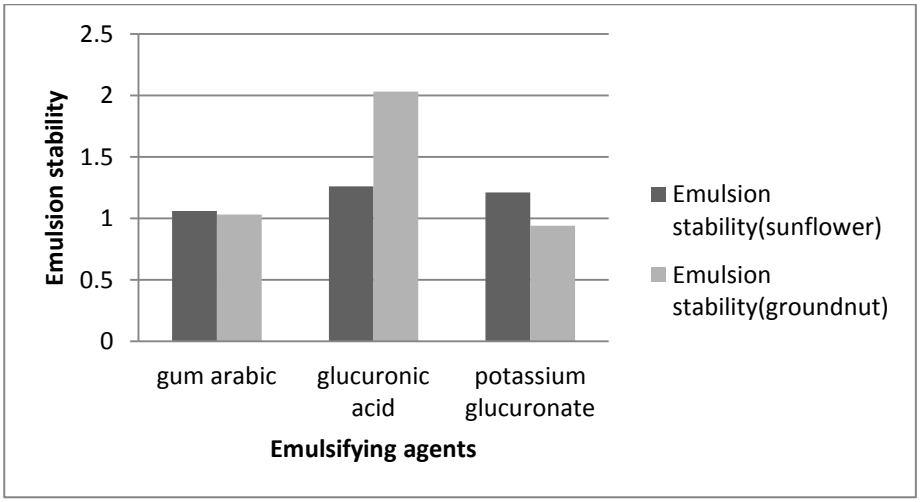

Fig 3.5: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate as affected by different oil types after three days using UV measurement

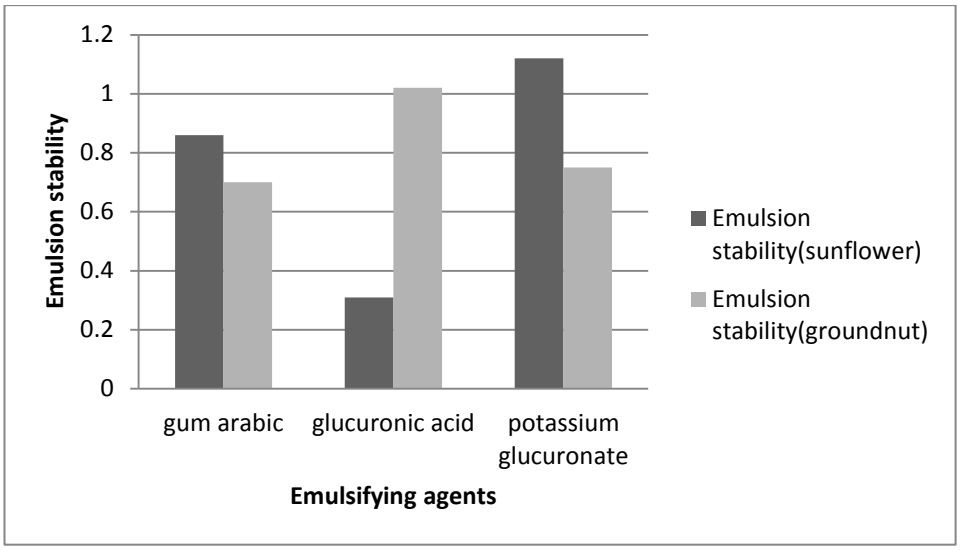

Fig 3.6: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate as affected by different oil types after five days using UV measurement

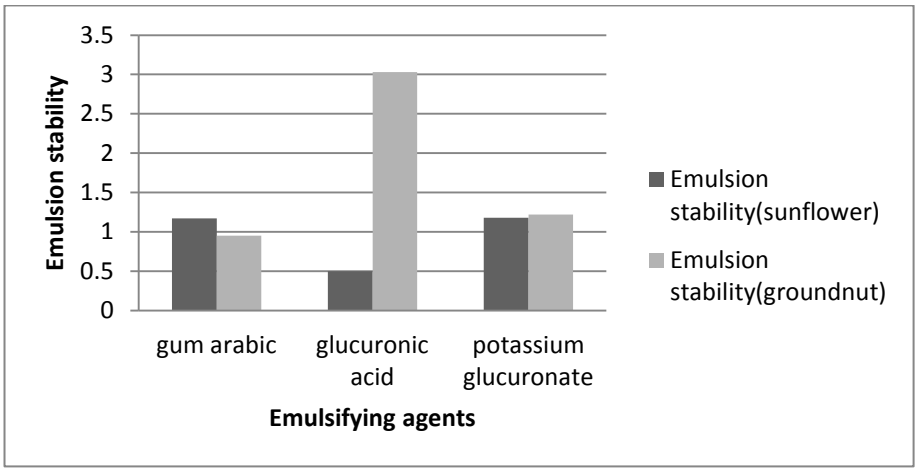

Fig 3.7: Emulsions stability of gum arabic, glucuronic acid and potassium glucuronate as affected by different oil types after seven days using UV measurement

From results of turbidity and absorption measurements it was found that *Acacia senegal* and potassium glucuronate had more stable emulsions than glucuronic acid. The explanation for the stability of *Acacia senegal* gum and potassium glucuronate refer to the *Acacia senegal* gum has a functional ability to act as an emulsifier that stabilizes oil-in-water emulsion (Yokoyama *et al*., 1988; Randall *et al*., 1988). It is now known that the protein-rich high molecular mass component adsorbs preferentially onto the surface

of the oil droplets. It is envisaged that the hydrophobic polypeptide chains adsorb and anchor the molecules to the surface while the carbohydrate blocks inhibit flocculation and coalescence through electrostatic and steric repulsions (NGARA, 2005), and the potassium glucuronate containing hydroxyl groups, the packing of the emulsifying agent molecules need not be altered because the polar metallic ions, and also the polar hydroxyl groups dip into the water giving ability of good emulsion; in addition it is probable that the presence of so many polar groups in the molecule makes the stability of emulsions of oil- in-water possible.

Emulsion prepared by sunflower oil is more stability than the emulsion prepared by groundnut oil. Differences in stability may be ascribed to differences in properties of the oil

3.3.Conclusions and Recommendations

Conclusions

On the basis of results obtained, it could be concluded that:

- The glucuronic acid is more acidity pH (2.7) than *Acacia senegal* pH (4.6) and potassium glucuronate pH (6.8).
- The glucuronic acid and potassium glucuronate have an intrinsic viscosity of 10.8 and 12.9ml/g respectively, while the *Acacia* gum has 15.3ml/g.
- The glucuronic acid and potassium glucuronate have the same specific optical rotation (levorotatary) for *Acacia* gum but with lower numerical values.
- The glucuronic acid and potassium glucuronate have the total ash content of 0.3%, 7.7% respectively, while the gum has 3.2%.
- *Acacia senegal* gum and potassium glucuronate gave more stable emulsions than glucuronic acid.
- The type of oil effected of emulsion stability, sunflower oil is gave a most stable emulsion than groundnut oil.

Recommendations

- Potassium glucuronate is recommended to for the preparation of stable emulsions.
- This study can be applied to another type of gums.

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