Sudan University of Science *and* Technology

College of Graduate Studies

Preparation and characterization of physicochemical and Functional Properties of Potassium Glucuronate from *Acacia seyal var. seyal* Gum

تحضيروتوصيف الخواص الفيزيوكيميائية والوظيفية لجلوكويورنات البوتاسيوم من صمغ الاكاشيا سيال صنف السيال

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الآيه

قال الله تعالى :

(قل هل يستوي الذين يعلمون والذين لا يعلمون انما يتذكرأولو الألباب)

(الزمر الآية 9)

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 friends and family for their support and help.

ABSTRACT

Acacia seyal var. seyal gum from Al Gadaref was used in this study. Physical and chemical properties such as moisture, ash, pH value, intrinsic viscosity, Molecular weight, specific rotation, nitrogen and protein, acid equivalent weight, total glucuronic acid, and cationic composition were determined for *Aseyal* gum. The values of moisture and ash found to be 11.09%, 2.77 % respectively, while the specific rotation value of the sample was +56.59˚. Intrinsic viscosity of sample was 14.7ml/g, while the calculated Molecular weight value from Mark-Houwink equation was 7. 5x10^5. The pH value was 4.4. while nitrogen and protein content 0.09%. ,0.528 %. The values of acid equivalent weight and percentage of total glucuronic acid were found to be 1587 and 11.5 % respectively. Results indicate that the calcium is major element present in *Acacia seyal* gum(2028.809ppm) when was compared with potassium(554.685), sodium(25.147) and magnesium(1120).

Glucuronic acid was prepared from *Acacia seyal* gum by cation exchange chromatography. Potassium glucuronate was prepared by titrating glucuronic acid with potassium hydroxide. Glucuronic acid and potassium glucuronate gave pH values of 2.9,7.8 respectively. The values of moisture were 6.2%, 7.1% respectively, while the viscosities were 10.3, 12.39ml/g. The values of ash were found to be 0.38, 5.6% respectively. The specific rotation value of glucuronic acid and potassium glucuronate were +59.934˚, +55.00˚ respectively. In addition the stability of *Acacia seyal*, 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 type of emulsifier and the type of oil used.

المستخلص

في هذه الدراسة استخدم صمغ الاكاشيا سيال من القضارف.تم تحديد الخواص الفيزيائية والكيميائية مثل الرطوبة، الرماد، قيمة الاس الهيدروجيني، اللزوجة الضمنية ، الوزن الجزيئي، الدوران النوعي، النتروجين و البروتين، الوزن المكافئ الحامضي،حامض الجلوكيورنيك الكلي وتركيب المعادن للصمغ العربي. وجد انقِيَم الرطوبة والرماد 11.09% و2.7% على التوالي،. بينما وجد ان قيمة الدوران النوعي للعينه °56.59+. اللزوجة الضمنية للعينه كانت 14.7مل/جرام، بينما قيمةِ الوزن الجزيئي المحسوبه من معادلة مارك كانت .7 $5x10^5$. كما وجد ان قيمة الاس الهيدروجيني 4.4. بينما محتوى النتروجين0.09 %. قيمة البروتين كانت 0.528 %. قيم الوزن المكافئ الحامضي والنسبة المئوية لحامض الجلوكيورنيك الكلي 1587،11.5 % على التوالي. تشير النتائج ان الكالسيوم عنصر رئيسي موجود في صمغ الاكاشيا سيال (2028.809 ppm) عندما قورن مع البوتاسيوم(554.685)، الصوديوم(25.147) والماغنسيوم(1120).

حضرحامض الجلوكيورنيك من صمغ الاكاشيا سيال باستعمال كروموتغرافيا التبادل الآيوني. جلوكويورنات البوتاسيوم حضر بمعايرة حامض الجلوكيورنيك مع محلول هيدروكسيد البوتاسيوم. أعطى حامض الجلوكيورنيك وجلوكويورنات البوتاسيوم قيم اس هيدروجيني 2.9،7.8 على التوالي. وجد ان قيم الرطوبة 6.2 %،7.1% على التوالي، بينما قيم الرماد 0.38, 5.6% على التوالي.كما وجد ان اللزوجة 10.3, 12.3مل /جرام. وجد ان قيمة الدوران النوعي لحامض الجلوكيورنيك وجلوكويورنات البوتاسيوم°59.934+ ,°55.00+ على التوالي. بالاضافه الي ذلك تمت دراسة إستقرارية مستحلبات اكاشيا سيال، حامض الجلوكيورنيك وجلوكويورنات البوتاسيوم باعتبار نوع الزيت (عباد الشمس والفول السوداني). اوضحت النتائج أن إستقرارية المستحلب يتاثر بشكل ملحوظ بنوعالمستحلبِ ونوع الزيت المستخدم.

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Chapter One

Introduction and literature review

1.Introduction and literature review

1.1.introduction

Exudates gums are among the oldest natural gums used as thickening and stabilizing agent .Exudates gums are produced by many trees and shrubs as natural defense mechanism, particulary in semi arid regions of Africa (Renard et al.,2006). When the plants bark is injured, an aqueous gum solution exudes to seal the wound, preventing infection and dehydration of the plant. The solution dries in contact with air and sunlight, to form hard, glassy lumps which can easily be collected (Verbeken 2003).

1.1.2.Gum Arabic

Gum Arabic along –chain, high-molecular weight polymer that dissolves in water to give a thickening effect is one of the oldest food ingredients.

Acacia seyal var. seyal and *Acacia sensgal var. sensgal* are the two species of Acacia gum which constitute Gum Arabic. *Acacia seyal* is less valued than Acacia Senegal due to its poor emulsification properties, therefore, it is considered to be an inferior quality gum. Commercial gum Arabic may contain up to Ca 1% *A. seyal* as a contaminant.

Gum Arabic is a complex mixture of polysaccharides, protein and arabinoglacto protein(AGP) species. It has been shown to be highly heterogeneous and is found in nature as mixed Ca, Mg, K, and Na salts of a polysaccharic 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.

1.2.literature review

1.2.1.Definition of gum Arabic

Gum Arabic definitions is based on the American Food Chemical Codex, published in 1969; WHO, 1969), the joint

Expert Committee for Food Additives (JECFA) of the FAO/WHO monograph on gum Arabic in 1978 (JECFA, 1978), which has been reviewed in four years (1982, 1986, 1990, 1995). In 1990 (JECFA. 1990), significant changes were made to definitions e.g. ranges for specific rotation (-26 to -34) and nitrogen content (0.27 to 0.39%) were introduced However, in 1995 JECFA, further recommended that specific rotation and nitrogen content are to be deleted from the definitions.

In (1993) Philips and William suggested that characterization of gum Arabic is possible using four parameters, e.g. specific rotation , viscosity, lysine and hydroxyproline composition. In 1996 (European Union, 1996) introduced the molecular weight limits.

In 1997 *A. seyal var. seyal* was accepted as closely related species (FAO, 1997). In 1998 Codex Alimentrarius Meeting, the JECFA proposed specification for gum Arabic, prepared at the JECFA meeting (1997), due to objection from Sudan, was sent back to JECFA for further consideration. In spite of objection to including of *A. seyal* gum in the specification of the gum Arabic. Another recommendation for the specification of gum Arabic, where *A. seyal* as gum Arabic, has been adopted, but gums from other Acacia species are not included in these specification.

In March 1999 the Codex Committee for Food Additives and Contaminants gave acceptance to the specification in category 11 (recommended for adoption after editorial changes, including technical revisions).

1.2.2.The Gum Arabic Belt

The gum arabic belt in sudan extends across the central region, lies between latitudes 10-16, covering about 520,000Km^2, accounting for one fifth of the country's total area before separation. The area accommodates around one fifth of the population of the sudan and two thirds of its livestock population.

A. seyal trees are up to 17 m tall in sudan, with aflat top crown. It has a distinctive smooth powdery bark, from white to greenish yellow or orange red, with agreen layer beneath. In som population both red and yellow barked trees can be found. There are two varieties, differing primarily in whether or not pseudo-galls ("ant galls") develop and in bark colour. In *A. seyal var. seyal*, there are no pseudo-galls and reddish bark color prevails, although periodic bark exfoliation exposes a pale powdery surface which darkens slowly. In *A. seyal var.fistula* pseudo-galls are present and the powdery bark typically remains whitish or greenish-yellow.

1.2.3.Chemical Structure of gum arabic

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(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,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, 1989, and Phillips, 1988). Figure 1.2: Hypothetical structure of arabinoglactan-protein(AGP) obtained from gum Arabic.

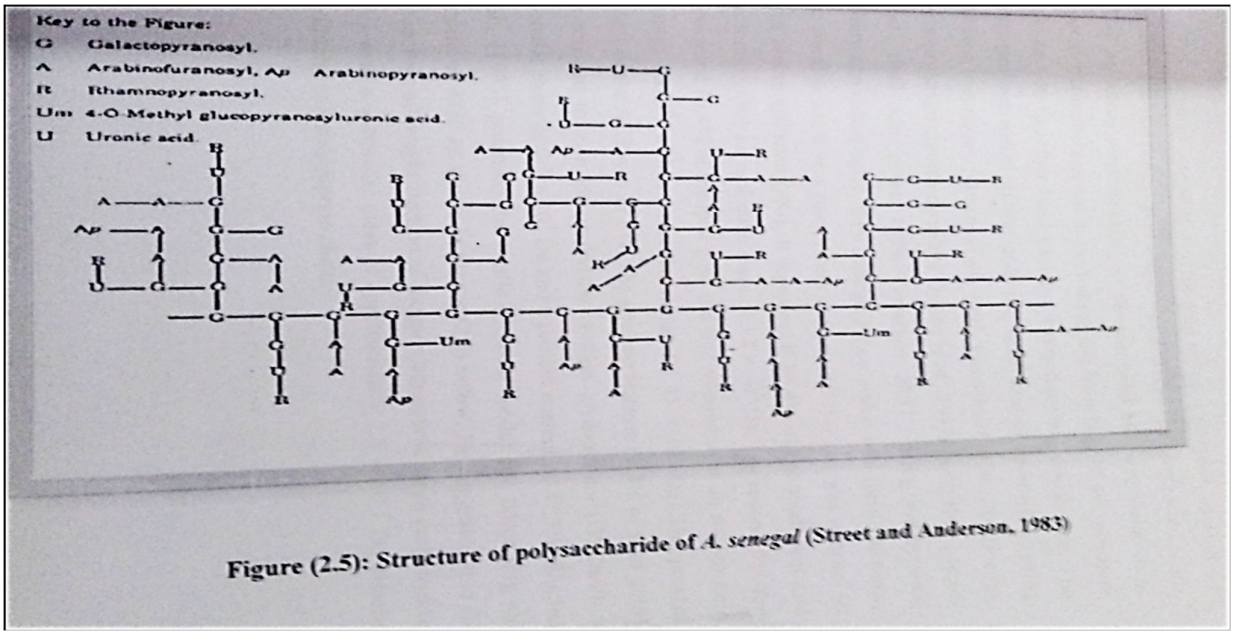

Figure (2.5): Structure of polysaccharide of *A. senegal* (Street and Anderson, 1983)

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

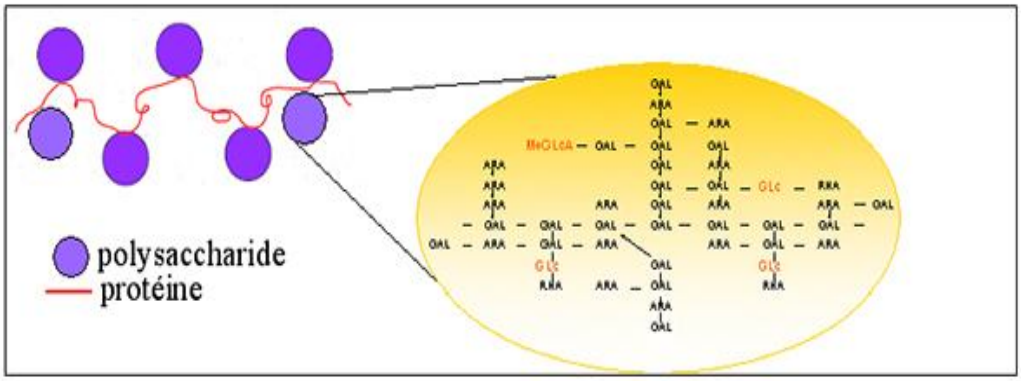

Figure 1.2: Hypothetical structure of arabinoglactan-protein (AGP) obtained from gum arabic

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 qupolysaccharide 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.

1.2.4.Collection and processing of gum arabic

Although natural exudates are sometimes harvested, virtually all exudate gum is 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 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. 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.

1.2.5. Physiochemical properties of gum Arabic

The physical properties of the natural gum 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 season of the year(Hirst *et al.*, 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.

1.2.5.1.Solubility

Acacia *tortilis* var. *raddiana* gum is highly soluble in water forming transparent solution, and classified as soluble gum.

1.2.5.2. Moisture content %

The moisture content of the *A. seyal A. seyal var. seyal or var. fistula* were falls within the range 7.2 – 16.3 %, while *A.senegal var. Senegal* falls within the range 7.4 - .15 %.

1.2.5.3. Ash content %

The Ash content of the *A. seyal A. seyal var. seyal or var. fistula* were falls within the range 0.7 – 3.61 %, while *A.senegal var. Senegal* falls within the range 2.0 – 3.70%.

1.2.5.4. Nitrogen and protein content

Nitrogen and hence protein content of the gum has been direct- related to its emulsifying stability (Dickinson *et al.*1988). Protein content was considered as one of the most important analytical and commercial parameters to differentiate between *A. seyal var. seyal* and *var. fistula* and *A. Senegal var. Senegal* in which nitrogen and hence protein content of *A. Senegal var. Senegal* gum account for, almost three folds of that of *A. seyal* from the two varieties.

1.2.5.5.Optical rotation

Specific rotation is one of the most important criteria of the purity and identity of gum Arabic. It acts as a unique parameter in physico chemical differentiation between *A. Senegal var. Senegal* gum and other botanically related *Acacia* gum. Almost all finding obtained by the authors stated that *A. seyal var. seyal or var. fistula* gum exhibit dextrogyrate specific rotaion, while *A. Senegal var. Senegal* gum exhibit laevogyrate specific rotation.

1.2.5.6.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 *et al.,* 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 e*t al.,* 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 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. Some of the Authors reported that intrinsic viscosity of the *A. seyal* (both varieties) ranges between 7.20 – 20.0 cm^-3 g^-1.

1.2.5.7. 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 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 are rapid, accurate and requires 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, 2000).

1.2.5.8. 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 the gum Arabic is called Arabic acid and hence.

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 value of 4.3 for pH of Acacia Senegal gum. Karamalla(1998) reported 4.66 pH values for *Acacia senegal* and 4.2 for *Acacia seyal* gum.

1.2.5.9.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

.Colorimetric Techniques:

Uronic acid onheating, 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.

.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 et al, 1963) and its infra red (IR) absorption at 2350nm 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.

.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%.

1.2.5.10. Cationic composition

This table shows that calcium, magnesium, sodium, and potassium are the most abundant elements in all seyal gum samples.

Sample Number	Ca (ppm)	Mg (ppm)	Na (ppm)	K (ppm)
1	10000	1230	750	2416
2	9800	650	878	2416
3	10000	650	666	3583
4	9615	650	N.D	2916
5	10000	823	250	2500
6	10000	500	250	2500
7	10750	883	250	2500
8	8333	1000	250	2416
9	9487	475	750	3083
10	10250	750	N.D	2500
Mean	9824	761	505.50	2683
S.D,	554.95	231.61	279.08	390.4
C.V,	5.65	30.43	55.21	14.55

Table 1.1: *Acacia seyal var. seyal* cationic composition(ppm)

C.D, coefficient of variation.

S.D, standard deviation.

1.2.6. Applications of plant gum

The solubility and viscosity of 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.

1.2.6.1. 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).

1.2.6.2. 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, some times in combination with other gums(Voget, 1995).

1.2.6.3. Paints and coating 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 *et al*., (1953) developed non8 glare coating based on a water soluble dye dissolved in gum Arabic solutions.

1.2.6.4. 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 and 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).

1.3. *Acacia seyal var. seyal*

1.3.1.Botanical Classification

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Subfamily	*Mimosoideate*
Order	*Fabales*
Family	*Fabaceae*
Genus	*Acacia*
Species	*A. seyal var. seyal*
Binomial name	*Acacia seyal*
Local name	Talha

1.4.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).

1.5.Emulsification properties of gum arabic, glucuronic acid and glucuronates

1.5.1.Definition of emulsion

An emulsion is a dispersed system that consist 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).

1.5.2.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(Mc Clements, 2007).

1.5.3. 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 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 changes in the gell permeation chromatography(GPC) profile of the gum.

He concluded that heating at 100ºC for 3 hrs, 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 *et al.*,(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 *et al.*,(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 also it gave the most stable n-hexadecane – water emulsion is smallest droplets with all three oils(n-hexadecane and orange oil). They also concluded that a high molecular weight fraction(0.87 nitrogen) corresponding 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).

1.5.4.Applications of emulsion

Emulsions can be used in variety of fields:

1.5.4.1.In food:

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).

1.5.4.2.In medicine

In pharmaceutics, hairstyling, personal hygiene, and cosmetics, emulsions are frequently used. These are usually oil in water emulsion, 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 5 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 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, so when they encounter the lipids to merge with themselves. On a mass scale, this effectively disintegrates the membrane and kills the pathogen. Soybean oil emulsion do 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.

1.6.Turbidity of Fluid

1.6.1.Definition

Turbidity is the measure of the "cloudiness" of water; more precisely, it measures the extent to which light is scattered and absorbed by 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.

1.6.2.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).

1.6.3. 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.

1.7. Spectrophotometery and spectroscopy

1.7.1. 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:

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

Where:

I_o is the incident light

I_t is the transmitted light

1.7.2. 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(RS232) interfacing. 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.

1.7.3. Objectives of study

1-To Determine the physicochemical properties of *Acacia seyal* 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 seyal* 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. 0.2 molar of sulfuric acid was passed through the column many times, the column was then washed with distilled water until no white precepted (BaSO4) by adding Barium chloride. 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 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.

2.2.4. 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 550ºC in muffle furnace (Nabertherm, Model B150) for 2 hours, cooled in a desiccater and weighed (W_3). Total ash% was calculated as follows:

$$\text{Total ash content \%} = \frac{W3-W2}{W2-W1} x100$$

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 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.

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

2.2.6.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 = α × 100/ C × 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.pH measurement

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

2.2.8.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).

2.2.9. 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).

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

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.

2.2.10.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.

2.2.11.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.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 seyal* 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 seyal*, 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 seyal* gum, glucuronic acid and potassium glucuronate

A number of physicochemical properties were used to characterize the studied sample of *Acacia seyal* gum, glucuronic acid and potassium glucuronate. Table 3.1 shows Physio-chemical Properties of *A. seyal var. seyal* gum, glucuronic acid and Potassium glucuronate. The moisture content was found to be 11.09%. While glucuronic acid and potassium glucuronate have the moisture content of 6.3%, 7.1%. Results show insignificant variation in the moisture content for parent gum. Ash percentage was found to be 2.7%.while glucuronic acid and potassium glucuronate have ash content of 0.38%, 5.6% respectively.

Table 3.1: Physio-chemical Properties of *A. seyal var. seyal* gum, Glucuronic acid and Potassium glucuronate

Property	*A seyal* gum	Glucuronic acid	Potassium glucuronate
Moisture %	11.09	6.288	7.1489
Ash %	2.77	0.3886	5.645
Nitrogen %	0.09	ND	ND
Protein %	0.594	0.594	0.594
Acid equivalent Weight	1587	1587	ND
Total glucuronic Acid %	11.5	11.5	ND
PH	4.4	2.9	7.8
Intrinsic viscosity (ml/g)	14.7	10.37	12.39
Molecular weight	7.5×10^5	ND	ND
specific Optical rotation	+56.59˚	+59.934˚	+55.00˚

ND = not determined

Ash% content indicates the presence of inorganic elements, Anderson and Dea(1968). FAO(1991) specifies that the total ash percentage as apurity test for gum Arabic should not exceed 4%. The results shown agreement with mentioned specifications. The pH of *Acacia seyal* gum aqueous solution was found to be slightly acidic(4.4) but glucuronic acid and potassium glucuronate have the pH value of 2.9, 7.8 respectively. Results show insignificant variation in the pH for parent gum.

A. seyal var. seyal sample has optical rotation value of +56.59˚. while the glucuronic acid and potassium glucuronate have the Optical rotation of +59.934˚, +55.00˚. Result indicate that the nitrogen content was 0.09%(0.594% Protein). In this study.

The equivalent weight value of 1587. Result obtained was within the range(1136-1875) reported by Karmalla et al(1998). The total uronic acid was found 11.5%. result obtained was within (10.34%-23.32%) reported by Karamalla et al.,(1998). But lower if compared to the value of 17% obtained by Jurasek(1993).

The *Acacia seyal* has Intrinsic viscosity of 14.7ml g^{-1}. While glucuronic acid and potassium glucuronate have Intrinsic viscosity of 10.3, 12.3 respectively. The viscosity of glucuronic acid solution is decreased with the addition of metal ions and it was further decrease with increasing concentration of NaCl(Schleif, 1951). The results are in good agreement with these reported by Schleif. the viscosity of the potassium glucuronate higher if 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 food and nutrition paper 52(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 seyal* gum sample (ppm)

Element	Na	K	Ca	Mg
Concentration/ppm	25.147	554.685	2028.809	1120

Table 3.2: shows that calcium, magnesium, potassium and sodium are the major elements in *A. seyal var. seyal* sample. Results obtained the Ca is major element present in sample(2028.809 ppm) and Na is less element present in sample(25.147 ppm).

3.2.Emulsification properties of *Acacia seyal* gum, glucuronic acid and potassium glucuronate

The emulsion 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, & 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 & 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(J. Bibitte, 1992). Many attempts have been made to make emulsion stable, such as, by using emulsifier, salts and polymers(A. L. 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(T. H. M. Snoeren, 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 type using turbidity measurement are shown in Fig: 3.1, 3.2, and 3.3.

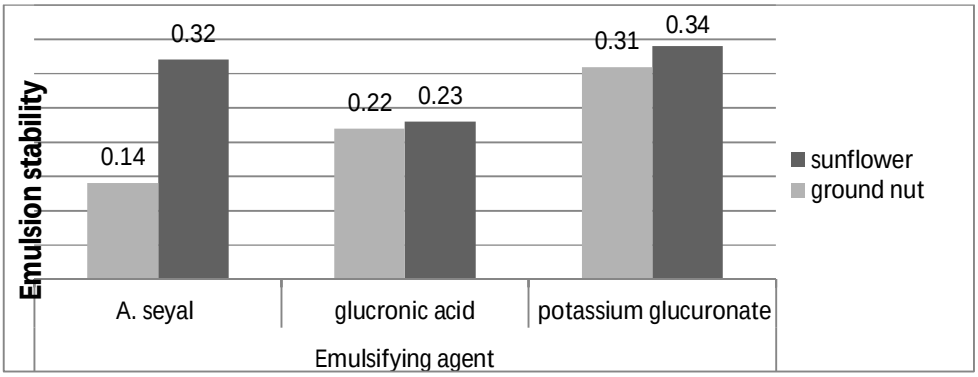

Fig 3.1: Emulsions Stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate as affected by different oil type after one day using turbidity measuremen

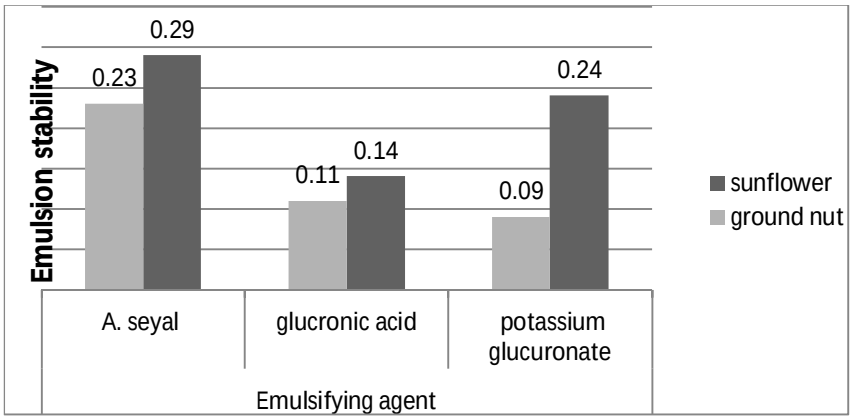

Fig 3.2: Emulsions Stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate as affected by different oil type after three days using turbidity measurement

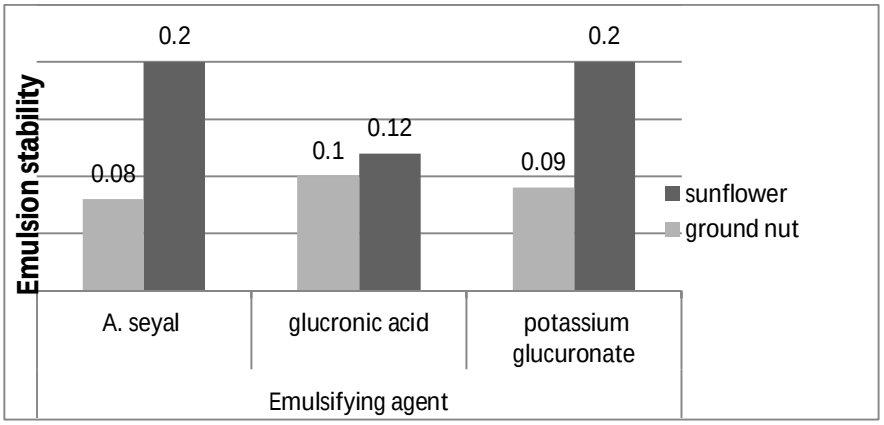

Fig 3.3: Emulsions Stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate as affected by different oil type after seven days using turbidity measurement

-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 seyal* and potassium glucuronate have a good emulsification properties if compared to the glucuronic acid.

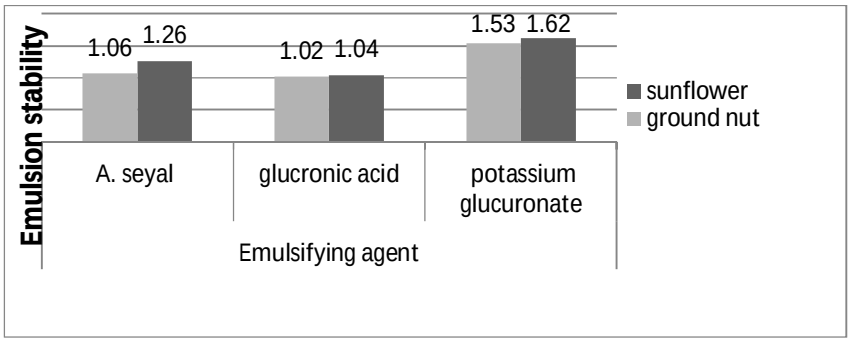

Fig 3.4: Stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate emulsions as affected by different oil type after one day using UV measurement

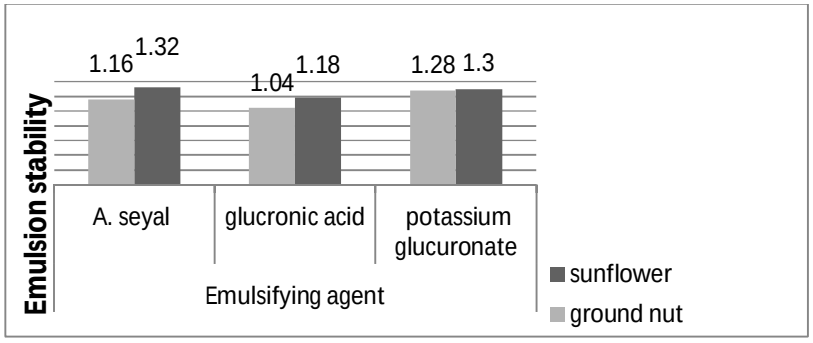

Fig 3.5: Emulsions Stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate as affected by different oil type after three days using UV measurement

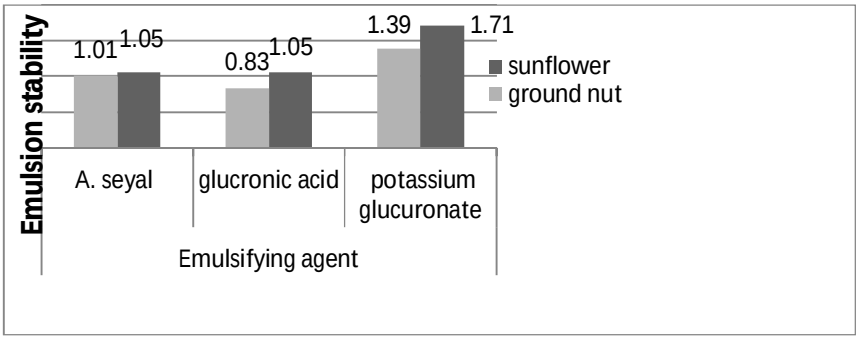

Fig 3.6: Emulsions stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate as affected by different oil type after five days using UV measurement

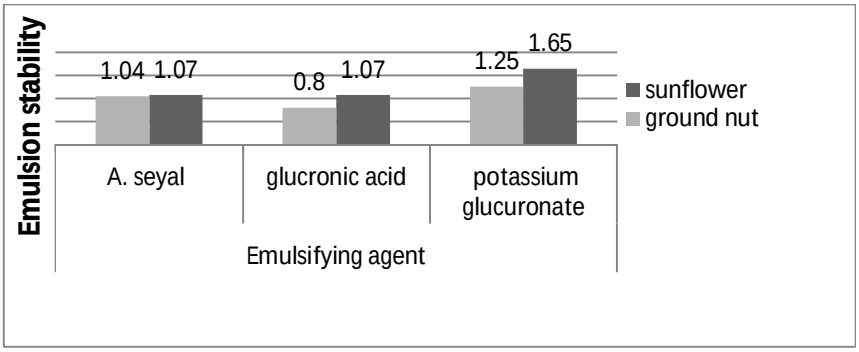

Fig 3.7: Emulsions stability of *A. seyal var. seyal* gum, glucuronic acid and potassium glucuronate as affected by different oil type after seven days using UV measurement

From results of turbidity and absorption measurement was found:

Acacia seyal gum and potassium glucuronate are more stable emulsion than glucuronic acid. The explanation for the stability of *Acacia seyal* gum and potassium glucuronate refer to the *Acacia seyal* gum has a functional ability to act as emulsifier that stabilizes oil-in-water emulsion(Yokoyama et al., 1988; Randall et al., 1988). It is now know 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 atoms, 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 production 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 difference in properties of oil

3.3.Conclusions

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

- Glucuronic acid is more acidic(2.9) than *Acacia seyal* (4.4) and potassium glucuronate(7.8).

-Glucuronic acid and potassium glucuronate have the Intrinsic viscosity of 10.3, 12.3ml/g respectively, while the *Acacia* gum has 14.7ml/g.

- Glucuronic acid and potassium glucuronate have the same Optical rotation for *Acacia* gum.

- Glucuronic acid and potassium glucuronate have the ash content of 0.38%, 5.6% respectively, while the gum has 2.7%.

- *Acacia seyal* gum and potassium glucuronate are 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.

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