CHAPTER ONE
GENERAL INTRODUCTION

1.1. Introduction

Exudates gums are among the oldest natural gums they were already being used as thickening and stabilizing agents about 5, 000 years ago.The three major, traditional, exudates gums are gum arabic, gum tragacanth, and gum karaya they possess a unique range of functionalities, that make them important items of international trade in the Food, Pharmaceutical, Adhesive, Paper, Textile, and other industries for centuries (Verbeken *et al*., 2003). *Acacia* gums are, natural, exudates polymers, produced by over 1000 species distributed on tropical and subtropical areas of Australia, Africa, India and Central America. Some of them are, commercially, important.

The important production areas of the African gum belt are Sudan, Chad and Nigeria. Sudan is largest producer in the world. However, the production of gum arabic in Sudan declined from 80% to around 60% of the world market sale in the last two decades (Anon., 2008).

Gum arabic is defined by the FAO/WHO (1998) Joint Expert Committee for Food Additives (JECFA) as: dried exudates obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (fam. Leguminoseae). It consists mainly of high molecular weight polysaccharides obtained as a mixed calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid (Al – Assaf, 2007). The research done on different types of

Acacia gums especially on *Acacia senegal* (Osman, 1993; Al – Assaf, 2007; Islam *et al*., 1997etc.), all of them mentioned that gums composed of mixture of some metallic salts of glucuronic acid and protein complexes. The main minerals are: calcium, potassium, magnesium and sodium. There are other minerals but they are present as traces. Regarding to the commercial interest and importance of the gum as food additive, physicochemical characterization of it is of great importance. According to the literature review, few studies have been presented glucuronic acid and glucuronates (Nazaik, 2003; Jayme *et al*., 1999).

1.2. Objectives

The objectives of this study are:-

- To authentic samples by determining their physicochemical properties.
- To modify gum arabic molecule using glucuronic acid to form individual glucuronate salts.
- To study the morphological feature (shape) of *Acacia senegal* gum, glucuronic acid and glucuronates using Scanning Electron Microscopy (SEM).
- To investigate the effect of chemical modification on viscosity and electrical properties (conductivity) of glucuronic acid and glucuronates.
- To investigate the effect of chemical modification on emulsification properties of glucuronic acid and glucuronates.
- To evaluate the rheological behavior of glucuronic acid and glucuronates.

CHAPTER TWO

LITERATURE REVIEW

2.1. Exudate gums.

Gums arabic are viscous liquid that oozes from the stems and branches of *Acacia* trees. There are over 1,000 species of *Acacia* but only the gum from *Acacia senegal* and *Acacia seyal* is referred to as gum arabic. The gum is produced when the trees are subjected to unhealthy conditions such as heat, drought or wounding. The liquid dries in the sun to form glassy nodules, which are collected by hand (Williams *et al*., 2006).

2.1.1. Definitions of gum arabic

As a matter of facts through history, gum arabic acquired different names due to the location and sometimes due to the color of it. For instance European gave it the name gum arabic, because it arrived to them via Arabian ports and also named Turkey gum and others local names (Verbeken *et al*., 2003). All regulatory definitions of gum arabic recognize that the gum arabic of commerce originates from more than one species of *Acacia*. According to the *US Pharmacopoeia* (1985), *US Food* and *Drug Administration* (*USFDA*, 1996) and FAO/WHO Joint Expert Committee for Food Additives *(JECFA*, 1999); *Acacia* gum or (gum arabic) is defined as: a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) *Willdenow* or *Acacia seyal* (family: *Leguminoseae*).

Acacia gum is defined as a heteropolysaccharide since it contains about 2% of a polypeptide. *Acacia* gum is also described as heteropolymolecular, because it has, on one hand, a variation in monomer composition or in the linking and branching of the monomer units and on

the other hand, a molecular mass distribution. The consequence of this heterogeneity was reflected through the molecular species collected after fractionation of the gum and the mode of both separation and detection used (Renard *et al*., 2006).It consists mainly of high molecular weight polysaccharides obtained as a mixed calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid (Al-Assaf *et al*., 2007).

2.1.2. Gum belt

The genus *Acacia* is the second largest within the *Leguminosae* family and contains at least 900 species, with their extensive root system. *Acacia* trees can be found in semi-arid areas in Australia, India, and America, but mainly in the Sahelian region of Africa. They are multi-purpose trees, not only producing gum, but also preventing desert encroachment, restoring soil fertility, and providing fuel and fodder. Almost all commercial gums come from the so-called gum belt of Africa. Figure (2.1) is a Map of Africa show gum arabic belts which are a vast area extends over Mauritania, Senegal, Mali, Burkina Faso, Benin, Niger, Nigeria, Chad, Sudan, Eritrea, Ethiopia, Somalia, Uganda, and Kenya. (FAO, 1995). Sudan is the world's largest producer of gum arabic, followed by Nigeria, Chad, Mali, and Senegal. Gum from the Sudanese Kordofan region is known as the best quality gum and is used as the standard to judge gums obtained from other areas. The term gum arabic belt is used to denote a zone of approximately 200,000 square miles which extends across central Sudan between latitude (10^{o} and 14^{o}) north, and which accounts for roughly one-fifth of the country's total area. The belt accommodates around a fifth of the population of the Sudan and two thirds of its livestock population (IIED and IES, 1990).

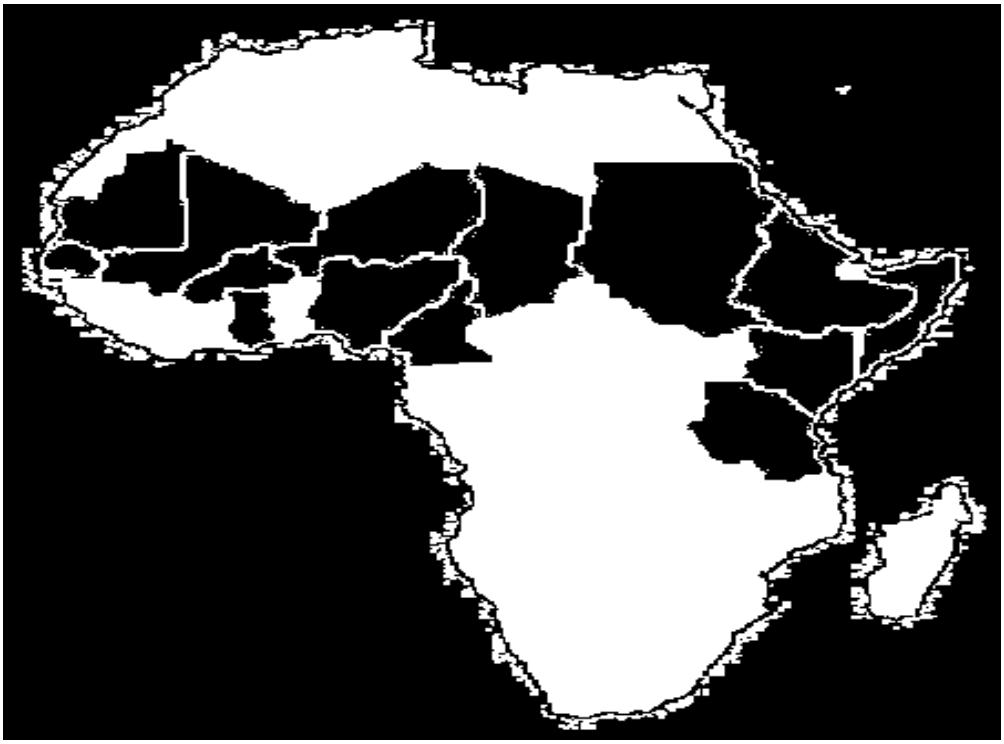


Figure (2.1): African gum belt

The belt is of a site for intense, diverse and often conflicting human activities. These include irrigate agriculture, mechanized and traditional rain fed agriculture, forestry and grazing. The gum belt was affected by many factors at last two decades such as, desertification due to the climatic change and frequent drought incidents, desertification due to the action of the local inhabitants, and changes in ocean temperature caused by the global warming might be the main culprit and these factors lead to destruction of the vegetation in Sudan, and destruction of the gum belt, this lead to the belt shift to south and east south wards.

The traditional gum belt in Sudan (Before the last two decades) cover about 9 states, namely, west Darfur, south Darfur, north Darfur, north Kordofan, west Kordofan, south Kordofan, Gadarif, Kassala, and white Nile, Gum belt in Sudan now companies 10 states divided to the

west regions and east regions, west regions the traditional source of gum arabic in Sudan, include west Darfur, south Darfur, north Darfur, north Kordofan, west Kordofan, and south Kordofan. East region the new plantation includes Sinnar, Blue Nile, Gadarif, and Upper Nile. In general there is clear environmental variation between the two regions such as soil type, rainfall, and the highest of the trees. Two areas are outside the border of traditional gum belt, in the south east (Blue Nile), and in the south (Upper Nile), this due to the shift of the gum belt during the last two decades, towards the south and south east (Anon., 1970 – 2008).This shift is corresponded to the shift of the rainfall belt and desertification engorgement in Sudan in the same directions. Due to the shift of the belt there is a new areas are joined to the belt (Blue Nile and Upper Nile), and now a day upper Nile produce between 30% - 40% of Sudan production(Gum Arabic company, 2005-2006), and also there is areas are eliminated from the gum belt (Kassala, white Nile, and a part of north Kordofan) which located at the north and north east , and we can take this also as evidence of the shifting of the gum belt towards south in Sudan. We might say currently the actual areas of gum production which compose the gum belt in Sudan is between latitude (10^o and 13^o or 12^o 50 north) approximately. Survey carried by the international institute of environment and development, and the institute of environmental studies (IIED) and (IES) 1990, suggest that the belt moved south wards and no acacia trees existing north of latitude 13^o 45 north. Figure (2.2) show the areas of gum production in Sudan which compose what called gum belt.

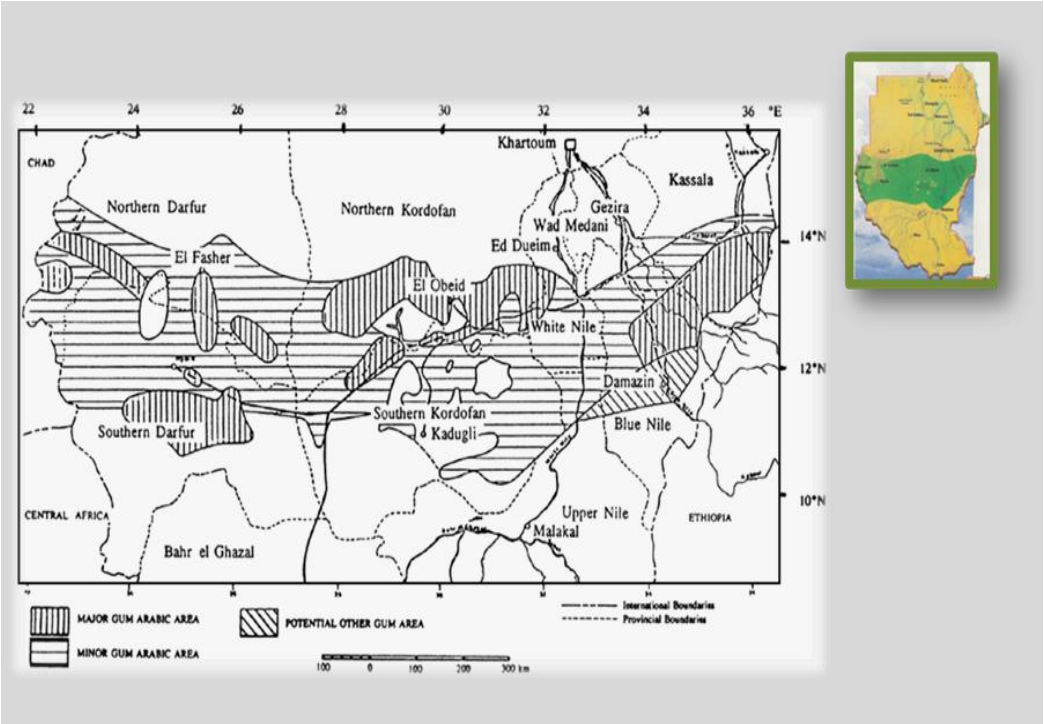


Figure (2.2): Sudan gum belt

2.1.3. Classification of gum arabic

The genus *Acacia* was taxonomically classified by Bentham (1875). He subdivided the genus into six series on the basis of geographical distribution, habitat and inflorescence. These series are: *Phyllodineae, Botrycephalae, Juliflorae, Gummiferae, Vulgares.* The three series mentioned first are found in Australia. The subgenres *Gummiferae* and *Vulgares* are found in tropical and semi-tropical geographical location (Al-Assaf, 2003). According to Bentham's taxonomic classification of the *Acacia* genus, *A. senegal* belongs to series 5 (*Vulgares*).

Series 5 VULGARES Benth. = Subgen.ACULEIFERUM Vas. Also called "*Acacia senegal* complex" that contains: *A.berlandieri, A. polycantha subsp. campylacantha, A. catechu, A. erubescens, A. fleckii,*

A. goetzii subsp. goetzii, A. laeta, A. mellifera, subsp. detinens, A. senegal, A.sundra.

2.1.4. Description of gum arabic trees.

Gum arabic is usually a small tree or a large shrub with low branching and short pole. The plant is readily recognized by the triple spines at the base of branch lets. The back is grey fissured and flakes off in papery patches. The growth slash is fibrous and exudes gums. The leaves are pinnate and alternate with 3-6 pairs of slender pinnate on a slender common stalk. Several pairs of narrow, linear –oblong leaflets exist. The flowers are cream – colored, fragrant and arranged in spikes, 5 –10 cm long while the fruits are contained in flattened pods with straight edges as shown in Figure (2.3) below. Sometimes, they are slightly constricted between the seed and are greenish – brown in color (Abubakar, 2004).The most common species of promising economic value are *Acacia senegal* gum and *Acacia seyal* gum.

Figure (2.3): Gum arabic

2.1.5. Classification of *Acacia senegal* (hashab)

Family: *Leguminosae*

Subfamily: *Mimosoideae.*

Genus: *Acacia*.

Subgenus: *Aculeiferum*.

Species: *senegal*

Series*: Vulgares*

2.1.6. Description of *Acacia senegal* tree

Gum arabic from *Acacia senegal* is a pale to orange-brown colored solid, which breaks with a glassy fracture. The best grades are in the form of whole, round tears, orange-brown in color and with a matt surface texture as presented in Figure (2.4) below. In the broken, kibbled state, the pieces are much paler and have glassy appearances. *Acacia senegal* is a xerophytic plant and grows in dry habitats namely Sudan Savannah and Sahel Zones. The plant tolerates water deficit and therefore able to endure conditions of prolonged drought in some places in the Sudan. It can be identified by its morphological features (Abubakar, 2004). The rooting habits of the *Acacia* species are especially suited to making maximum utilization of available moisture. Germinating seeds develop a long tap root reaching about two meters in two months, then growth of lateral roots develop in the second and third years. This branching and far rooting enables the young seedling to continue to grow even after the rains have stopped. Trees are tapped when they reach the height of about 1-1.5 meters, after a period of 3-7 years depending on the method of establishment (seed or plant). The two first years represent the period when the *Acacia* tree is in the greatest danger from animals or neglect, before it can be cropped. The acacia tree reaches full maturity at 20-25

years old, but there are many authenticated examples of trees over 40 years which are still capable of producing gum. In general, production seems to slow down after the age of 25 years.

Figure (2.4): *Acacia senegal* tree

2.1.7. Production of gum arabic in Sudan

Gum is one of the main products of the traditional rainfall sector in Sudan. Sudan is endowed with more than 30 *Acacia* species, most of them yield gum (EL-Amin, 1990), and species with greatest distribution include *Acacia senegal* (hashab), *Acacia seyal* (talha), *Acacia Polyacantha* (kakamout), *Acacia laeta* (shubahi), *Acacia Mellifere* (kitir), *Acacia Nilotica* (sunt), *Acacia sieberiana* (kok). The biggest producers of gum are *Acacia senegal*, *Acacia seyal*, and *Acacia Polyacantha* respectively (Anon., 1970 – 2008).

There is an evidence indicate that the north area of the gum belt has been decreased, and current gum production mainly comes from the

southern and southern east parts. In modern times, Sudan has dominated the production and trade of gum arabic, accounting for 80–90% of the world market (Chikamai *et al*., 1996). Sudanese production and export data is therefore representative for the whole market. Table (2.1), below gives an overview of the production of gum arabic in Sudan for the period 1960–1994.

Table (2.1): Gum arabic production in Sudan (5-year annual averages), between 1960 and 1994 (FAO 1999)

Period	Annual average (ton)
1960 – 1964	46.550
1965 – 1969	50.576
1970 – 1974	35.073
1975 – 1979	37.408
1980 – 1984	31.079
1985 – 1989	23.721
1990 – 1994	18.358

In the late 1960s, Sudanese gum arabic production was in excess of 60.000 t / year; but in the 1970s and 1980s the gum market was disturbed by climatic and political events. In 1973–1974, a severe drought occurred in the Sahelian gum belt, resulting in the collapse of gum production to 20.000 t/ year. Together with the dramatically increased prices and the fact that the decrease in supply was not announced, the limited availability caused a major loss of confidence by manufacturers, many of whom switched to alternative materials, such as modified starches and dextrins (Imeson,1992). The Sudanese gum production recovered to

around 40,000 t at the end of the 1970s, but a new drought in 1982–1984, together with political and civil unrest, brought production down to below 20,000 t at the end of the 1980s. In the early 1990s, gum supply further decreased due to cold weather, rainfall fluctuations and foliage attack by locusts, and changes in export duties. Over recent years, production strongly recovered to an estimated 40,000–50,000 t/year (Williams and Phillips 2000; Williams *et al*., 1990). These important supply variations led to strongly fluctuating prices. After the first drought, the gum price increased dramatically from about US$ 1,500/t to US$ 5,000/t and even up to US$ 8,000/t. Now days, prices are back at the same level as 30 years ago.

2.1.8. Collection and processing.

Although natural exudates are sometimes harvested, virtually all exudate gum is tapped from the trees. 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. 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 (Imeson1992). 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 convey or belts and sieving machines. In Sudan, the gum from *Acacia senegal* (hashab) is presented in various grades, listed in Table (2.2). 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 (2.2): Commercial grades of *Acacia senegal* gum from Sudan (Islam *et al*. 1997)

Grade	**Description**
Hand-picked selected	The 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 other lumps.

2.2. Chemical structure of gum arabic

Gum arabic is a branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salt. The backbone consists of 1, 3 -linked β-D -galactopyranosyl units. The side chains are composed of two to five 1, 3 -linked β-D -galactopyranosyl units, joined to the main chain by 1, 6-linkages. Both the main and the side chains contain units of α-L-arabinofuranosyl, α-L -rhamnopyranosyl, β-D -glucuronopyranosyl, and 4-O-methyl β-D -glucuronopyranosyl, the latter two mostly as end-units (Verbeken *et al*., 2003).The characteristics may vary significantly, depending on the geographical origin and age of the trees, climatic conditions, soil environment, and even on the place of exudation of the trees.

All *Acacia* gums are arabiogalactan-protein complexes. The carbohydrates represent more than 95% of the gum (Shimon 2006). Carbohydrate portion is composed by D-galactose, L-arabinose, L-rhamnose and two uronic acids (D-glucuronic acid and 4-O-methyl-D-glucuronic acid).Three major molecular species have been isolated, an arabingalactan, an arabinogalactan- protein complexe and aglycoprotein (Randal,1989).

The carbohydrate structure has shown that it consists of a core of β (1, 3) linked galactose units with extensive branching at the C6 position. The branches consist of galactose and arabinose and terminate with rhamnose and glucuronic acid .There is a low portion of protein, containing high levels of hydroxyl proline (Al-Assaf, 2008). Figure (2.5) below show structure of polysaccharide of *A. senegal* as presented by Street and Anderson (1983).

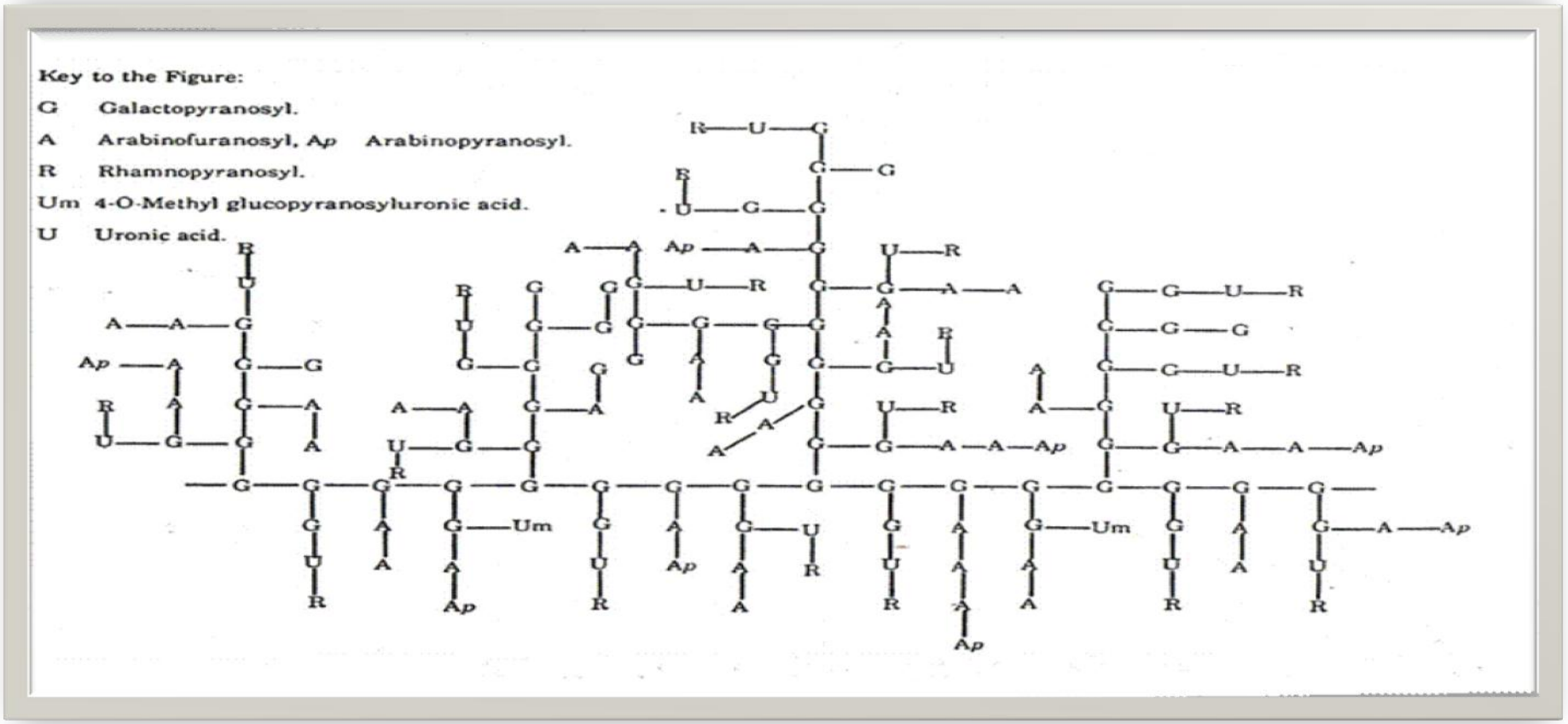


Figure (2.5): Structure of polysaccharide of *A. senegal* (Street and Anderson, 1983)

The chemical composition of the gum arabic is complex, because, of its heterogeneous nature, gum arabic was described by (Anderson ,*et al*., 1966) as hetero polymolecular, on the one hand having a variation in monomer composition and in the linking and branching of the monomer units and on the other hand having a molecular mass distribution. The heterogeneous nature of the gum has been studied extensively using different techniques. (Osman *et al*., 1995; Fauconnier *et al*., 2000) using anion-exchange chromatography,(Osman *et al*., 1993a and 1993b ; Islam *et al*., 1997; Idris *et al*., 1998; Verberken, 2003) using hydrophobic affinity chromatography, they separated gum arabic into three fractions; first fraction has a very low protein content (0.35%) and was referred to as an arabinogalactan (AG). It represented 88.4% of the total gum and was found to have a molecular mass of 3.8×10^5 Da. The second fraction represented 10.4% of the total gum and was referred to as an AG–protein complex (AGP) with a molecular mass of 1.45×10^6 Da. The protein content of the AGP was 11.8%. The smallest fraction (1.2% of total gum) was referred to as a low molecular- weight glycoprotein (GP) with a molecular mass of 2.5×10^5 Da and a protein content of 47.3%. Fincher *et al*., (1983) proposed the wattle blossom model for describing the structure of AGP as shown in Figure (2.6) below. He suggested that the high-molecular-weight fraction of the gum consists of large carbohydrate blocks with a molecular mass of approximately 2.5×10^5 Da attached individually to a polypeptide chain.

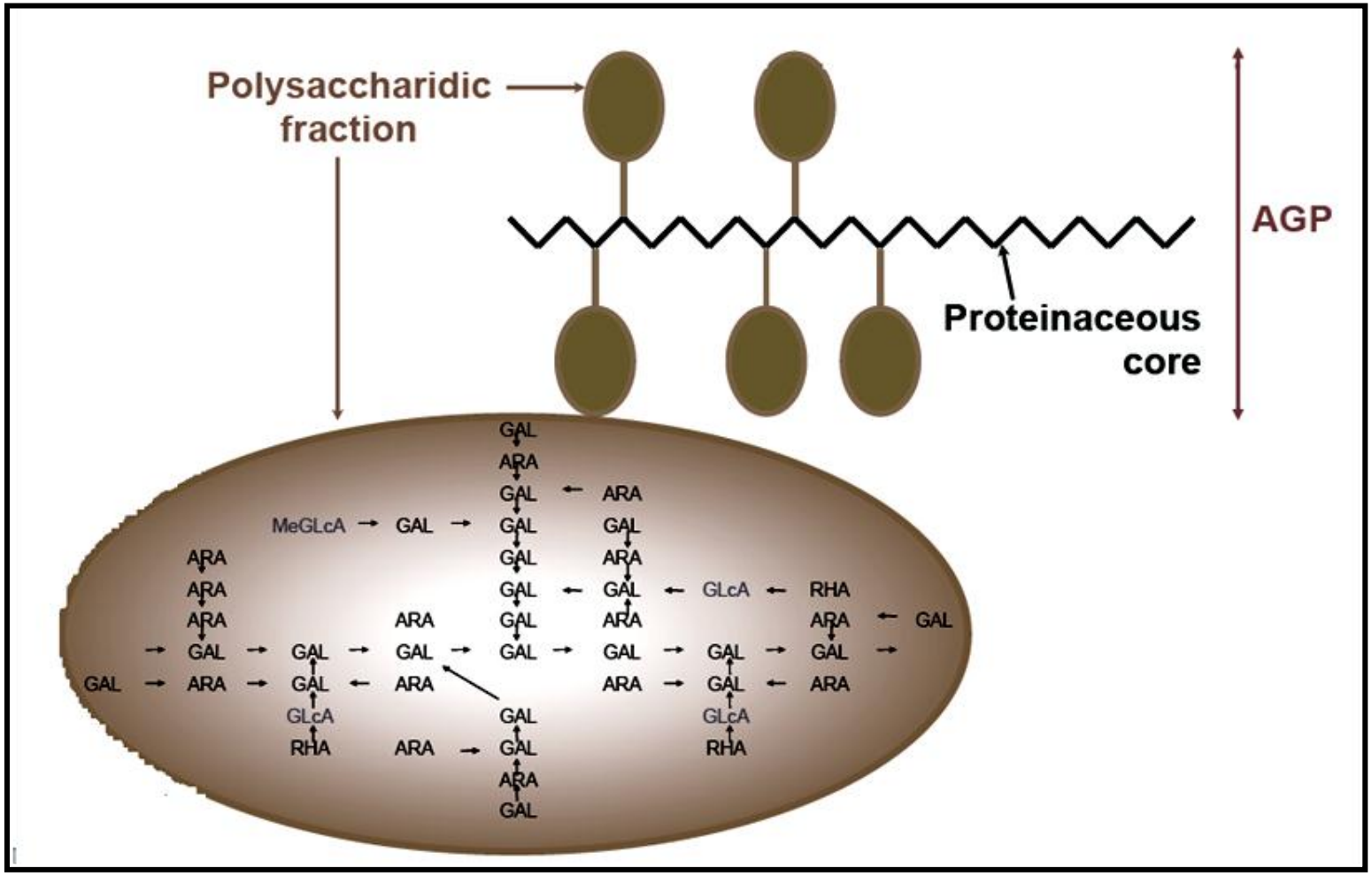


Figure (2.6): Wattle blossom model of arabinogalactan–protein (Fincher *et al*, 1983)

An alternative model was proposed by Qi *et al*., (1991), who described the AGP as a twisted hairy rope as shown in Figure (2.7), using chromatographic techniques and electron microscopy , he concluded that the molecules had a polypeptide backbone of some 400 amino acid residues with numerous small carbohydrate substituent's (molecular mass ~6,000 Da) linked through hydroxyproline. Over the years, numerous studies were conducted, suggesting that AGP molecules have a globular structure and thus supporting the wattle blossom model (Idris *et al*., 1998; Vandevelde *et al*., 1985). The polypeptide backbone of the gum arabic GP is composed of repeating sequences of 19 amino acids.

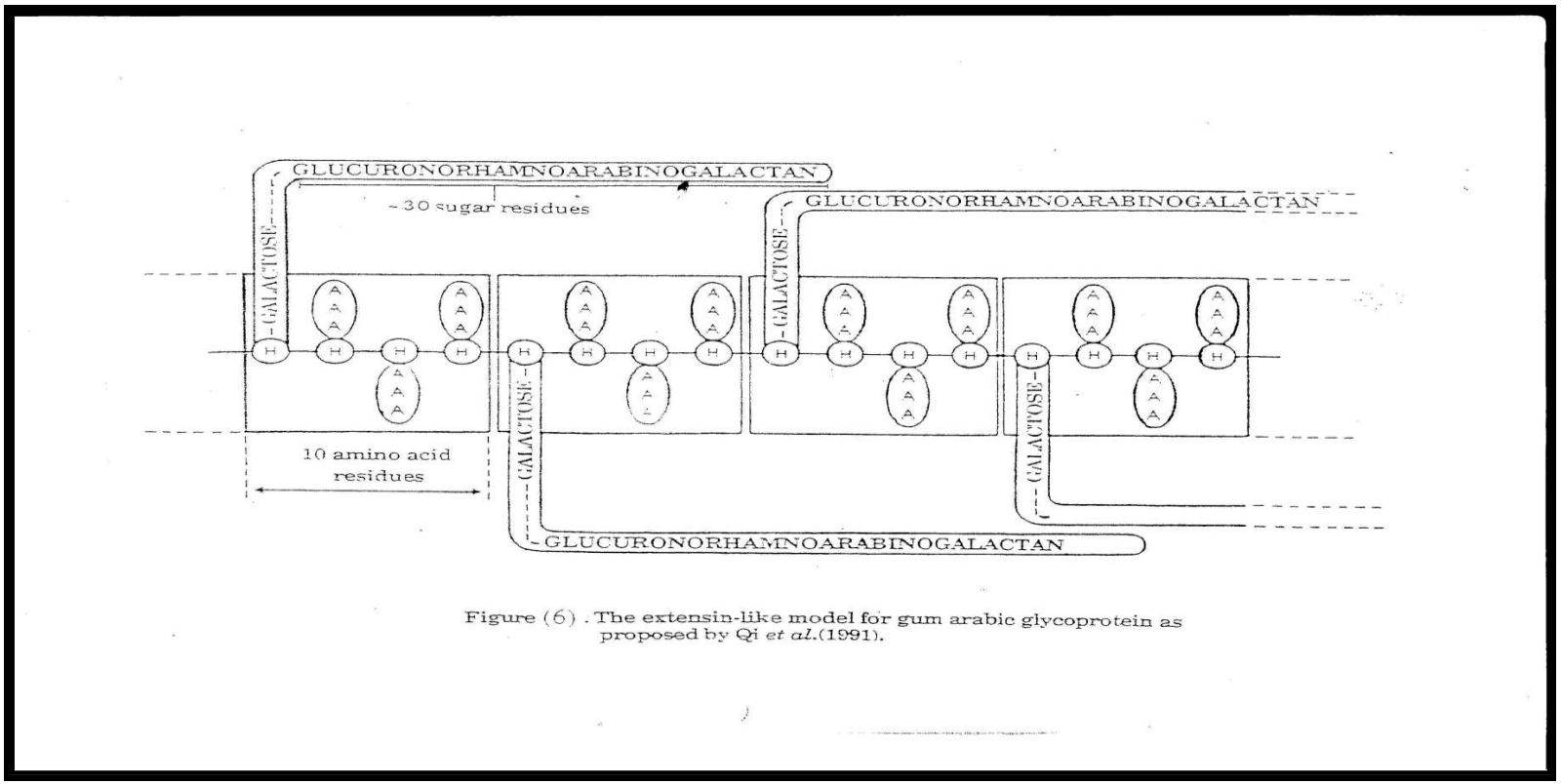


Figure (6) . The extensin-like model for gum arabic glycoprotein as proposed by Qi *et al.*(1991).

Figure (2.7): Twisted Hairy Rope model for the high molecular mass fractions of *Acacia senegal* gums (Qi *et al*., 1991)

Generally, *Acacia seyal* gum is less valued than *Acacia senegal* gum due to its poor emulsification performance (Flindt *et al*., 2005) and, therefore, tends to be used for applications where long term emulsion stability is not required (Elmanan, 2008).

Acacia seyal gum has a higher weight average molecular weight than that of *Acacia senegal* gum, and that this is mainly due to greater proportions of the high molecular weight components, as identified by various fractionation methods. Unusually, this higher molecular weight is not reflected in a higher intrinsic viscosity for *Acacia seyal* gum relative to *Acacia senegal* gum; this would indicate that the molecular structure is more compact in *Acacia seyal* gum than in *Acacia senegal* gum. Hydrophobic interactive chromatography (HIC) identified a high molecular weight protein rich component (AGP), a lower molecular weight (AG) component with low protein and a third component in *Acacia seyal* gum of high protein content and high molecular weight. It is probable, therefore that the high molecular weight material identified by gel permeation chromatography is made up of at least two separate components (Flindt, 2005).

Acacia seyal gum was fractionated on the basis of molecular size and hydrophobicity in to a number of fractions. The molecular weight of the whole gum and its fractions were determined using gel permeation chromatography (GPC) with on-line monitoring using light scattering,
refractive index and UV absorbance detectors. The results show that the three main components designated arabinogalactan protein (AGP),
arabinogalactan (AG) and glycoprotein (GP) known to be present in *Acacia senegal* gum could also be present in *Acacia seyal* gum. However, in *Acacia senegal* gum, the high molecular weight AGP component is more significant and is present in greater proportion than for *Acacia seyal* gum. The

most important difference is that at least two components are present in the high molecular weight fraction in *Acacia seyal* gum, indicating that it would be erroneous to identify this completely with the AGP fraction in *Acacia. senegal* gum (Siddig, *et al*., 2005).

The specific optical rotation of *Acacia seyal* gum is positive but negative for *Acacia senegal* gum. This difference has been shown recently to be due to differences in the proportion of the various sugars present. There is more arabinose and less rhamnose in *Acacia seyal* gum (B. Biswas, S. Biswas *et al*., 2000; Biswas *et al*., 2003).There is some evidence that *Acacia* s*eyal* gum is more compact in structure than *Acacia senegal* gum (Anderson *et al* 1968; Street & Anderson, 1983). Smith periodate degradation indicates that only a few of the amino acids in *Acacia seyal* are attached to periodate vulnerable sugars in peripheral positions and that the majority of the amino acids are in deep-seated locations within the structure (Anderson *et al*., 1987). There is approximately half the amount of protein in *Acacia seyal* gum compared with *Acacia senegal* gum in a different taxonomic series (Gummiferae) to *Acacia senegal* (Vulgares).

2.3. Physicochemical properties of gum arabic

Physicochemical properties are very important in determining the commercial values and end uses of such products as gum arabic .The most important measurable properties of gum arabic are:

2.3.1. Solubility

It is highly soluble in water. It yields a solution up to 50% concentration, and at this level it forms high- solid gels. It is insoluble in most organic solvents. Limited solubility also is obtained with ethanol (up to 60%), glycerol and ethylene glycol.

2.3.2. Moisture content

Moisture content express the amount of water present in a moist sample. It can be expressed on wet or dry basis. Moisture content on dry basis is the amount of water per unit mass of dry solids in the sample. Karamalla *et al*., (1998) reported the mean value of moisture content for 803 *Acacia senegal* gum samples collected in season 1994/1995 was 10.75% and the range was 8.1% – 14.05%. Younes (2009) reported the mean value of moisture content for *Acacia senegal* gum as 11.01% and the range was 9.91% – 14.72%, and the mean value of moisture content for *Acacia seyal* gum was 10.10% and the range was 9.90% – 10.35%. Anderson and Herbich (1963) reported the mean value of moisture content of *Acacia seyal* gum to be range from 11.0 -16.1%. Hassan (2000) in the study of *Acacia seyal* gum from different locations of Sudan reported an average of 8.5% moisture content; he also in (2005) reported the moisture content of *Acacia seyal* gum in the range from 7.4% to 8.3%.

2.3.3. Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating, which provides a measure of the total amount of minerals within a food. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components. The three main types of analytical procedure used to determine the ash content of foods are based on this principle: dry ashing, wet ashing and low temperature plasma dry ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available. Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods describe.

Anderson (1977) reported the ash content of *Acacia senegal* and *A. seyal* gum in the value of 2.87% and 3.93% respectively. Jurasek *et al.*, (1993) found that the ash content of *Acacia senegal* gum was 3.0%. The mean value of ash content had been determined for 731 *Acacia senegal* gum samples collected in season 1994/1995 by Karamalla *et al*., (1998) and was found in the range of 2.75 – 5.25%. Omers (2006) reported the ash content of *Acacia senegal* gum in an average of 3.27% and in an average of 2.61% for *Acacia seyal* gum .The mean value of ash content reported by Abdelrahman (2008) in *Acacia senegal* and *Acacia seyal* gum is in the average of 3.32% and 2.43% respectively. The total ash content of gum arabic *Acacia senegal* was reported to be 3.56% (Yebeyen, 2009).

Anderson and Herbich (1963) reported the values of 1.94 - 3.55% for the individual nodules of *Acacia seyal* gum. Anderson and Weiping (1991c) reported the range of 1.6 – 3.9% ash for *Acacia seyal* gum. Hassan *et al*., (2005) in the study of *Acacia seyal* gum from different locations of Sudan reported an average of 0.21% ash content.

2.3.4. Specific optical rotation

The specific rotation of a chemical compound [α] is defined as the observed angle of optical rotation α when plane-polarized light is passed through a sample with a path length of 1 decimeter and a sample concentration of 1 gram per 1 milliliter. It is the main property used to quantify the chirality of a molecular species or a mineral. The specific rotation of a pure material is an intrinsic property of that material at a given wavelength and temperature. Values should always be accompanied by the temperature at which the measurement was performed and the solvent in which the material was dissolved. Often the temperature is not specified; in these cases it is assumed to be room temperature. The formal unit for specific

rotation values is deg. $dm^{-1}cm^3\ g^{-1}$ but scientific literature uses just degrees (Mohrig *et al*., 2010). Substances that have the power of rotating the plane of polarized light through a certain angle are termed optically active. If the rotation is in clockwise direction the substance is called dextrorotatory (positive rotation) and if it is anticlockwise the substance is called laevorotatory (negative rotation). The extent of rotation of the plane of the polarized light can be measured by a polarimeter. The optical activity of organic molecules (saccharrides and carbohydrates) is related to their structure and is a characteristics property of the substance, and thus the specific rotation is considered as the most important criterion of purity and identity of any type of gum. Table (2.3) give analytical data for authentic *Acacia senegal* var *senegal* gum samples collected in season 1994/1995 (Karamalla *et al*., 1998)

Vavdevelde and Fenyo (1985) reported specific optical rotation of *Acacia senegal* gum to be ranging between -29^o to -34.4^o. FAO (1990) reported the value of specific optical rotation to be in the range of -26° to -34° for *Acacia senegal* gum. Jurasek *et al.*, (1993) reported the specific optical rotation of *Acacia senegal* gum to be range from -20° to -32° and a value of +51^o for *Acacia seyal* gum. Osman *et al*., (1993) reported specific optical rotation of *Acacia senegal* gum to be ranging between -29^o to -31^o. Karamalla *et al*., (1998) reported the specific optical rotation for the 789 authentic *Acacia senegal* gum samples, between -26^o to -34^o. Optical rotation of *Acacia senegal* gum samples collected from trees of various ages and different locations by Idris *et al*., (1998) was found to be in the range of -27^o to -36^o. Karamalla (1999) reported -30.3^o specific rotation for *Acacia senegal* and +50.6^o for *Acacia seyal* gum. Abdelrahman (2008) reported the average value of optical rotation of *Acacia senegal* gum to be -31.5^o. Anderson *et al*., (1963) reported the specific optical rotation of *Acacia seyal* gum in the range from +44^o to

+56^{o}, he also in (1977) reported a value of -30^{o} specific optical rotation for *Acacia senegal* and +51^{o} for *Acacia seyal* gum. Hassan (2005) reported +53^{o} mean value of specific optical rotation of *Acacia* s*eyal* gum.

Table (2.3): Analytical data for authentic *Acacia senegal* var *senegal* gum samples collected in season 1994/1995 (Karamalla *et al*., 1998)

Variables	**No. of samples**	**Minimum**	**Maximum**	**Mean**	**S.L.**	**C.V.**
Moisture%	803	8.1	14.05	10.75	1.128	10.5
Ash %	731	2.75	5.25	3.77	0.679	17.99
Nitrogen%	642	0.225	0.425	0.328	0.055	16.93
$[\alpha]_D$	789	-23	-39	31.8	3.63	11.59
pH	755	4.3	5.1	4.66	0.251	n.d.
Intrinsic viscosity	94	1	37	16.44	n.d.	100.65
M_v	95	3.1×10^{5}	7.7×10^{6}	9×10^{5}	n.d.	174.09
Equivalent weight	115	1136	1875	1436	144	10.03
Uronic anhydride%	11.5	10.34	23.32	13.71	1058	11.52

n.d. = not determined

2.3.5. Viscosity

The viscosity of a liquid is its resistance to shearing, stirring or to flow through a capillary tube. Viscosity was considered as one of the most important analytical and commercial parameters, since it is a factor involving the size and the shape of the macro – molecule (Anderson *et al*., 1969). Although gum Arabic is ahigh molecular weight it is a high soluble gum, it has a rather low viscosity, higher viscosity is not obtained with gum until the concentration of about 40 – 50 % (Glicksman, 1969). It can be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic viscosity. It is also presented as kinematic or dynamic viscosity. The intrinsic viscosity has great practical value in molecular weight determinations of high polymers. This concept is based on the Mark-Houwink relation suggesting that the intrinsic viscosity of a dilute polymer solution is proportional to the average molecular weight of the solute raised to a power in the range of 0.5 to 0.9. Values of the proportionality constant and the exponent are well known for many polymer-solvent combinations. Solutions viscosities are useful in understanding the behavior of some polymers. The intrinsic viscosity can be obtained by measuring specific viscosities at different concentrations at the same shear-rate, and extrapolating the course of specific viscosity to infinite dilution. The intrinsic viscosity [η] is, therefore, obtained by extrapolating data to zero concentration by using a linear regression. McMillan (1974) showed that $\frac{\eta_{sp}}{c}$ could be written in the form of Huggins-equation

(Huggins, 1942):

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]\,2C \qquad (2.1)$$

Where k' is the Huggins constant. The determination of the intrinsic viscosity is therefore, the extrapolation of $\frac{\eta_{sp}}{C}$ to the value at zero solute concentration. The extrapolations are usually done for relative viscosity values between 1.2 and 2.0, the corresponding specific viscosities being between 0.2 and 1.0 (Da Silva & Rao, 1992). In addition, McMillan (1974) reported that the intrinsic viscosity could be obtained from the Kraemer's equation (Kraemer, 1938) by extrapolation to zero concentration:

$$\frac{In\eta_{rel}}{C} = [\eta] + k''[\eta]\,2C \quad \text{(2.2)}$$

Where k'' is the Kraemer constant. For very dilute solutions, however, eq. (2.2) can be shortened by retaining only the first-order term, and [η] can be determined from the slope of a plot of C against lnηrel. McMillan (1974) showed that methods of determination of the intrinsic viscosity that were based on slopes of plots had higher correlation coefficients and lower standard errors, compared with those based on intercepts of plots. On the basis of such findings, Tanglertpaibul and Rao (1987) used the following equations to obtain the intrinsic viscosity of tomato serum (Higiro, *et al*., 2006):

$$\eta_{rel} = 1+[\eta]C \quad \text{(2.3)}$$

The intrinsic viscosity [η] is the intercept obtained by plotting η_{rel} vs C.

$$\eta_{rel} = e^{[\eta]C} \quad \text{(2.4)}$$

The intrinsic viscosity [η] is the intercept obtained by plotting $ln\eta_{rel}$ vs. C

$$\eta_{rel} = 1/1–[\eta]C \quad \text{(2.5)}$$

The intrinsic viscosity is the intercept obtained by plotting

1- $\frac{1}{\eta rel}$ vs. C…… (2.6)

The intrinsic viscosity [η] was estimated based on the intercept of $\frac{\eta_{sp}}{C}$ vs. C for polyelectrolytes; this is similar to the method discussed in eq. (2.3) and when there is essentially no molecular interaction, as in dilute solutions, the second term of the Huggins equation (eq. 2.1) is negligible, and a plot of $\frac{\eta_{sp}}{C}$ against concentration is linear.

Anderson (1977) reported a value of 13.4 cm^3g^{-1} intrinsic viscosity for *Acacia senegal*. Duvallet *et al*., (1989) reported that the intrinsic viscosity of *Acacia senegal* had a value of 21.8cm^3g^{-1}. Jurasek *et al*., (1993) found that the intrinsic viscosity ranged between 13.4 - 23.1 cm^3g^1 for *Acacia senegal* and equal to 12.4cm^3g^{-1} for *Acacia seyal* gum. Idris *et al*., (1998) studied the intrinsic viscosity of *Acacia senegal* gum from trees of different ages and different locations and concluded that the intrinsic viscosity varies with age of the trees but no affect was seen from trees in different locations. He found that the intrinsic viscosity of *Acacia senegal* gum was in the range from 10.4 to 19.8 cm^3g^{-1}. Karamalla *et al*., (1998) reported that the mean value of intrinsic viscosity of 1500 samples of *Acacia senegal* gum was 16.44 cm^3g^{-1}. Also Karamalla (1999) reported the intrinsic viscosity was equal to 16.6 cm^3g^{-1} for *Acacia senegal* and 11.0 cm^3g^{-1} for *Acacia seyal* gum. Hassan *et al*., (2005) reported the intrinsic viscosity of *Acacia seyal* gum in the ranges between 11.9–17.6 cm^3g^{-1}. The intrinsic viscosity had been determined by Flindt *et al*., (2005), they reported that the intrinsic viscosity of *Acacia seyal* gum fall in the range from 11.6 to 17.7 cm^3g^{-1}. Al-Assaf *et al*., (2005) reported that the intrinsic viscosity of sixty seven samples of *Acacia senegal* gum in the range 9.7 - 26.5 cm^3g^{-1}. The intrinsic viscosity of *Acacia seyal* gum had been

determined by Siddig *et al*., (2005), it was found to be 14cm^3g^{-1}. Omer (2006) found that an average values of intrinsic viscosity equal to 14.6 cm^3g^{-1} and 11.4cm^3g^{-1} for *Acacia senegal* and *Acacia seyal* gum respectively. Abdelrahman (2008) reported the average value of intrinsic viscosity for *Acacia senegal* gum 15.4 cm^3g^{-1} where as equal to 11.6 cm^3g^{-1} for *Acacia seyal* gum. The intrinsic viscosity had been determined by Elmanan *et al*., (2008), they reported that the intrinsic viscosity ranged between 14.7 to 17.3 cm^3g^{-1} for *Acacia senegal* gum and between 14.6 to 14.9cm^3g^{-1} for *Acacia seyal* gum and Younes, (2009) reported a value of 18.9 cm^3g^{-1} intrinsic viscosity for *Acacia senegal* and 15.5 cm^3g^{-1} for *Acacia seyal* gum.

2.3.6. pH value

The qualitative determination of the pH value of foodstuffs is, probably, are of the oldest analysis method in the world. All foodstuffs are tested with the taste organs. There by, some are noticed to be acidic and some to be alkaline. With modern pH electrodes these taste sensations can be measured exactly. Whether something is perceived as acidic or alkaline depends on the hydrogen ion (H+) concentration in the solution. The pH value is defined, by the Sorenson Equation, as the negative logarithm of the H+ concentration in a given solution. In other words, at a high concentration, example 1 mol. /L = 100, pH = 0 (ACIDIC).

At a low concentration, $H^+ = 10^{-14}$ mol. /L, pH = 14 (ALKALINE). Hence, different substances are objectively compared with each other, where pH 0 is extremely acidic, pH 14 extremely alkaline, and pH 7 is neutral.

The hydrogen ion concentration plays an important role in the chemistry and industry of gums. The change in the concentration of hydrogen ion may determine the solubility of gum and the precipitation of protein, therefore functional

properties of a gum may be affected by change in pH for example viscosity and emulsifying power. Crude gum arabic is slightly acidic because of the presence of few free carboxyl groups of its constituent acidic residues, D-glucuronic acid and its 4-O-methy1 derivatives.

Karamalla (1965) reported pH values of 4.42 for *Acacia senegal* gum while he recorded value of 4.74 for *Acacia seyal* var. *fistula* gum. Anderson (1967) reported values of 4.3 for pH of *Acacia senegal* gum. Karamalla *et al*., (1998) reported the pH mean value of 4.66 for the 755 authentic *Acacia senegal* gum samples, collected in season 1994/1995. Karamalla (1998) reported 4.66 pH values for *Acacia senegal* and 4.2 for *Acacia seyal* gum.

2.3.7. Nitrogen and protein content

The importance information about the composition of food products has been reassessed due to its wide range of applications related to diet and nutrition programs, nutritional value labeling, nutritional education, and international trade, promotion of new crops and transformation and consumption of new edible species, among others.

The Kjeldahl technique is the method commonly used for nitrogen and protein analysis in food products. The original Kjeldahl method, developed more than 100 years ago but undergone numerous modifications (Rossi *et al.*, 2004).

A lot of researcher reported about the total percentage of nitrogen content of *Acacia senegal* gum. Karamalla (1998) reported the mean value of 0.328 nitrogen content for *Acacia senegal* gum. Idris *et al*., (1998), was reported the value to be from 0.22 - 3.90% in his study of *Acacia senegal* trees from different ages. Elmanan *et al*., (2008) was reported the total value to be range from 0.27 - 0.33%, Yebeyen *et al*.,(2009) in his study on *Acacia senegal* gum in central rift valley of

Ethiopia , he reported the value to be 0.35. For *Acacia seyal* gum Anderson and Herbich (1963) reported the value of 0.09 - 0.19%, where Flindt *et al*., (2005) and Anderson reported the value to be from 0.08 -0.38% of nitrogen content.

The protein content was calculated using a nitrogen conversion factor of 6.6 (Anderson, 1986). Table (2.4) presented analytical data for *Acacia senegal* and *Acacia seyal* gum samples (Elmanan, 2008).

Table (2.4): Analytical data for *Acacia senegal* and *Acacia seyal* gum samples (Elmanan, 2008)

Sample	Moisture (%)	Ash (%)	pH	Optical rotation	Nitrogen (%)	Protein (%)
A.senegal	11.7	3.4	4.4	-31	0.32	2.15
A.senegal	13.4	3.4	4.3	-33	0.27	1.83
A.senegal	12.2	3.2	4.4	-34	0.33	2.20
A.senegal	13.4	3.0	4.4	-31	0.30	2.01
A.seyal	11.9	2.5	4.3	+56	0.10	0.66
A.seyal	11.2	1.6	4.3	+52	0.12	0.79

2.3.8. Equivalent weight and Uronic acid content

The acid equivalent weight or the titrable acidity of the gum can be determined by converting the acids salts of a solution containing a known weight of the gum sample into free acids and titrating against standard alkali. Uronic acid

usually presents in gum as free glucuronic acid, free 4-O-methylglucuronic acid and their salts.

Gums were found to differ widely in their equivalent weight and uronic acid content (Anderson *et al.,* 1977). Karamalla, (1965) in a comparative study of sixteen *Acacia senegal* samples reported the 14-16% for uronic acid. Anderson *et al*., (1983) studying authenticated and commercial samples of *Acacia senegal* found that the authenticated samples gave 1100 equivalent weight and 16 uronic acid where the commercial samples gave 990 equivalent weight and 18% uronic acid. Jurasek (1993) reported an average value of 17% uronic acid for *Acacia senegal* gum solution. Idris *et al*., (1998), in his study of *Acacia senegal* reported the equivalent weight in the range of 1118 – 1238%. Karamalla (1999) calculated the glucuronic acid percentage for *Acacia senegal* gum in the range of 16-17%. While for *Acacia seyal* gum was in the range of 11-12%. For *Acacia seyal* Anderson and Herbich (1963) reported uronic acid among individual nodules to be 9.0-16.8%. Anderson and Weiping (1991c) reported the range of 10-15% uronic acid for 8 samples of *Acacia seyal* gum solutions from three African countries.

2.3.9. Number average molecular weight (M_n)

The number average molecular weight (M_n) determined by Osmometry. Osmosis is the phenomenon of the spontaneous passage of the solvent into solution or from more dilute to concentrated solution, when the two are separated from each other by a semi permeable membrane. This tendency is due to in equality of the chemical potential of the solvent in the two liquids since the mole fractions of the solvent are different. The osmotic pressure of a solution is the additional pressures which must be applied to the solution to make the chemical potential of the solvent in the solution compartment exactly the same as in the

pure solvent compartment or is the pressure which needs to be applied to solution to prevent the inward flow of water across a semi-permeable membrane.

The phenomena of osmotic pressure arise from the tendency of a pure solvent to move through a semi-permeable membrane and in to a solution containing a solute to which the membrane is impermeable (Voet Donald, 2001).

The osmotic pressure π , is given by Van' t Hoff (1887) formula, which is identical to the pressure formula of an ideal gas.

The Van't Hoff equation: $\pi /C = RT/ M_n$ ……. (2.7)

The first values reported in literature came from Oakley (1935, 1936) who obtained a number average molecular weight (M_n) of 101,000 and 217,000, by ultracentrifuge studies Saverborn (1944) found the values in the range of 256,000 – 326,000 on samples of gum arabic and its sodium and calcium salts. Swenson *et al*., (1968) found the osmatic pressure molecular weight of gum arabic to be 250,000 and the light scattering molecular weight to be 365,000. Duvallet (1989) obtained osmatic pressure molecular weight of gum arabic in 0.01M NaCl at 37^{o}c to be 1.9×10^5. Idris *et al*., (1998) obtained values between 1.6 x10^5 and 4.5 x 10^5 of the number average molecular weight (M_n) for *Acacia senegal* gum.

2.3.10. Refractive index

The refractive index or index of refraction of a substance is a measure of the speed of light in that substance. It is expressed as a ratio of the speed of light in vacuum relative to that in the considered medium. The velocity at which light travels in vacuum is a physical constant, and the fastest speed at which energy or information can be transferred. However, light travels slower through any given material, or medium, that is not a vacuum.

A simple mathematical description of the refractive n = speed of light in a vacuum / speed of light in medium.

As light exits a medium, such as air, water, or glass, it may also change its propagation direction in proportion to the refractive index

(Snell's law).By measuring the angle of incidence and angle of refraction of the light beam, the refractive index **n** can be determined as follows:

$$\mathbf{n} = \sin i / \sin r = v/v1 \quad \text{(2.8)}$$

Where:

n: index of refraction

i: angle of incidence

r: angle of refraction

v: speed of light in first medium

$\boldsymbol{v^1}$: speed of light in second medium

The value of refractive index reported by Anderson *et al.*, (1969) was 0.146cm^3/g for *Acacia senegal* gum and 0.149 cm^3/g for *Acacia seyal* gum where value of 0.141cm^3/g for *Acacia senegal* gum by Randall *et al*., (1989).

2.3.11. Sugar composition

Sugar components of gum arabic is determined by complete hydrolysis of gum arabic which yields four basic sugar constituents; D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. The proportions differ from species to species. Table (2.5) shows data of sugar composition obtained by some authors.

Table (2.5): Sugar composition of *Acacia senegal* and *Acacia seyal* gums

Species	Galactose (%)	Arabinoe (%)	Rhamnose (%)	Glucuronic acid (%)	References
A.senegal	41	27	14	14.5	Anderson (1977)
A .senegal	34 - 46	23 - 35	09 -16	-	Jurasek *et al* .,(1993)
A .senegal	35	27	14	21	Osman *et al*.,(1993)
A .senegal	44	27	13	14.5	Islam *et al*.,*(*1997)
A.senegal	39 - 42	24 - 27	12 - 16	-	Idris *et al.,* (1998)
A.senegal	36 - 42	24 - 29	12 - 14	16 - 17	Karamalla (1999)
A.senegal	29.7	21	10.1	-	Abdelrahman (2008)
A.seyal	38	46	4	-	Jurasek *et al.,* (1993)
A.seyal	38	46	4	6.5	Islam *et al*.,(1997)
A.seyal	28.5	34	1.6	-	Abdelrahman(2008)

2.3.12. Cationic composition

Atomic absorption spectrophotometry is powerful tool for measurement of trace metallic elements presents in a sample.The most four abundant cationic elements present in gum arabic are calcium, potassium, magnesium, and sodium. Other elements such as arsenic, cadmium, cobalt, iron, zinc, cobalt and molybdenum are present as traces. Table (2.6) below show data of cationic composition obtained by some authors.

Table (2.6): Cationic composition of *Acacia sensgal* gum samples (ppm).

Species	*A. senegal*	*A. senegal*	*A. senegal*	*A. senegal*	*A .senegal*	*A. seyal*	*A. seyal*
Mg	24000	39000	38000	1938-1345	267	11.7	27
Ca	206000	316000	256000	5387-6314	6490	11200	7000
K	1600	221000	237000	6664-7735	261	7900	10100
Na	8400	10200	940	3.84-11.99	266	5.49	9.67
Zn	9.0	40	24	0.24-0.38	-	620	13
Cu	32	66	52	1.14-1.45	-	130	51
Fe	54	110	128	2.48-6.85	-	-	190
Mn	03	57	106	2.37-8.76	-	750	200
Pb	0	11	6.0	< 0.84	-	-	-
Al	111	311	190		-	-	-
Cr	39	34	47	0.27-0.34	-	-	-
Ni	03	12	10	< 0.22	-	-	-
References	Anderson *et al* (1984)	Anderson *et al* (1989)	Anderson *et al* (1990)	Buffo *et al*. (2001)	Younes (2009)	Siddig (2003)	Siddig (2003)

2.4. Modification of gum arabic

2.4.1. Properties of gum arabic

Gum arabic readily dissolves in cold and hot water in concentrations up to 50%, because of the compact, branches structure and therefore small hydrodynamic volume, gum arabic solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications (Dziezak 1991). Solutions exhibit Newtonian behavior at concentrations up to 40% and become pseudoplastic at higher concentrations. The pH of the solutions is normally around 4.5–5.5, but maximal viscosity is found at pH 6.0.Gum arabic has excellent emulsifying properties, particularly thanks to its AGP fraction. The hydrophobic polypeptide backbone strongly adsorbs at the oil–water interface, while the attached carbohydrate units stabilize the emulsion by steric and electrostatic repulsion. Fractionation studies show that, although emulsifying properties generally improve with increasing molecular weight and protein content, the best results are obtained with mixtures of different fractions (Ray *et al*. 1995). Seemingly, the heterogeneous nature of the gum makes it an excellent emulsifier (Buffo *et al*. 2001); found that stability of beverage emulsions is influenced by a number of processing factors, such as pasteurization and demineralization, and by the pH of the emulsion.

2.4.2. 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 hr. 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 (Thomas *et al.;* 1928).

- 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^{o} yielded an insoluble material.

- Recentaly Schleif and Co-workers prepared glucuronic acid by ion exchange method and successfully dried it by spray-drying at 205^{o} to yield a fluffy, white, finely subdivided powder. A similar ion exchange method was used by Wood to prepared glucuronic acid. This procedure was also used by Swintosky and Co-workers for the preparation of glucuronic acid and several other polysaccharides acids (Wood; 1954).

2.4.3. Properties of glucuronic acid

glucuronic acid is moderately strong acid having a pH of 2.2-2.7 in aqueous solution. For a moderately concentrated solution, $K_a=2.0 \times 10^{-4}$ at 22^{o}, ranking it about the same as lactic as, stronger than acetic but weaker than the chloroacetic acids. The values of K_a varies with concentration and $K_a=1\times10^{-3}$ for a 5% solution and 2×10^{-7} at infinite dilution.

Glucuronic acid has an equivalent weight of about 1200 by various workers. Upon addition of acid, glucuronic acid shows very little change in pH .The addition of neutral salts lower the pH of glucuronic acid solution. glucuronic acid has lower viscosity than any of its salts or gum arabic itself. Its viscosity can be

lowered further by the addition of sodium chloride and lowered to an even greater degree by the addition of calcium chloride or similar divalent salts.

Emulsions prepared with glucuronic acid will cream rapidly and are not stable as those made with its glucuronates, divalent salts produce a greater decrease than monovalent salts. Pure glucuronic acid is non-reducing towards Fehling,s solution and has specific optical rotation of -27^{o} to -30^{o} in water (Glicksman and Ralph; 1959).

2.4.4. 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. However, the simultaneous exchange of cations and anions can be more efficiently performed in mixed beds that contain a mixture of anion and cation exchange resins, or passing the treated solution through several different ion exchange materials (Dorgner 1991).

2.4.5. Preparation of glucuronates

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

- Krantz and Gordon (1929) precipitated the calcium ions in glucuronic solution by the addition of sodium carbonate. The solution is then filtered and evaporated to dryness to yield sodium glucuronate.
- Briggs (1934) used direct neutralization of glucouronic acid solution with sodium hydroxide followed by evaporation to dryness under reduced pressure at 70^{o}. Similar methods have been used for preparing calcium glucuronate and other salts.
- Schleif, Higuchi and Busse (1951) prepared sodium glucuronate using ion exchange methods. Glucuronic acid prepared by ion exchanging was treated with 0.5*N* sodium hydroxide until a pH of 8 was reached. The solution was then spray-dried at 205^{o}C to give a fluffly, fine, white powder. This method was extended to prepare spray-dried glucuronates of potassium, calcium, magnesium, zinc, iron and aluminum. Wood 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. glucuronic acid was passage through a cation – exchange resin in the salt form, a high purity glucuronates is formed. Potassium, sodium, lithum and ammonium glucuronates were prepared

2.4.6. Effect of cations on viscosity of glucuronic acid

In order to interpret the effect of the cations on the viscosity of gum arabic and glucuronic acid it is necessary to review the viscosity studies made by Kruyt and Bungenberg de Jong (1949) on colloidal solutions.

It has been noted by these investigators that certain colloidal solutions which contain charged colloidal particles exhibit a peculiar type of curve when reduce viscosity was plotted against concentration. This behaviour is attributed to the charge on the molecule and is known as the electro-viscous effect. The influence of the ionic environment on the viscosity is a phenomenon very typical of solution of charged, solvated macromolecules. It is clear that in the presence of charges the skein will not assume its statistically most probable form, but will have a less dense form through the interaction of the charged spots (mutual repulsion), or a more compact form if positive and negative charges are present simultaneously. As a result, the viscosity, which is sensitive to the form of the skein, is one of the most important aids in the investigation of systems with charged macromolecules. If there are only charged spots of one sign on the molecule, then the molecule will be relatively spread out. If charged spots of both signs are present, then the molecule will be more compact than corresponds to the most probable state. This remodelling of the skein not only depends on the interaction of the charged spots, but also on the extension of the ion atmosphere around each charged spot. The shape of the molecule will therefore also depend on the concentration and nature of different electrolytes in the solution, since these determine the thickness of the ion atmosphere.

When specific viscosity/concentration is plotted against concentration, Kruyt and Bungenberg de Jong (1949) have observed for agar solutions and other colloids that as the concentration are decreased the curve decreases to a minimum,

then at low concentrations a rapid rise occurs again. They called the viscosity due to the charge the electro-viscous effect. It was also noted by them that electrolytes added to the colloidal solutions will suppress this charge, resulting in a curve which is linear. For colloidal solutions with a negative charge the addition of an electrolyte with a divalent cation will result in a greater suppression of the charge, and a trivalent cation would suppress it further. Suppression of the charge results in a more compact molecule; therefore, some of the water surrounding the molecule is squeezed out, resulting in less hydration and lowered viscosity. Self-suppression of the charge by the colloidal particles at a higher concentration has been given as the explanation for the minimum in the curve discussed above.

Gum arabic, from which the glucuronic acid for the investigation was derived, is an example of a colloid with a negative charge. This charge is due to the carboxyl groups in the molecule. It would be expected, therefore, that a charge would exist on molecules of glucuronic acid and its glucuronates as it does on gum arabic (Schleif, 1951)

2.4.7. Equivalent conductivity of gum arabic, glucuronic acid and glucuronates

By the term polyelectrolyte is meant a high molecular weight compound which contains a number of ionizable groups distributed along the polymer chain. Polyelectrolytes, unlike their analogs the simple electrolyte on the one hand, and the neutral polymers on the other, show some characteristic behaviour in solution, e.g., viscosity, conductivity, osmatic pressure etc. These peculiar behaviours of polyelectrolytes arise, according to Fuoss, due to presence of high charge densities on the polymer chain in dilute solution and flexibility of the polymer chain. If the degree of ionization of the Polyelectrolyte chain is decreased by any factor, say by an increase in concentration or by adding a suitable electrolyte, the average

distance between the charges centres in the polymer chain increases; consequently the columbic repulsion between the charge centres decreases and the polymer chain coils up Basu (1951). It has been shown that gum arabic disperses to molecular units in solution and colloidal character of the solution is associated with the colloidal dimension of the molecule itself. If the gum arabic molecule constituent units are arranged in a chain with the carboxyl group of the uronic acid distributed along the chain, the sodium salt of the acid in dilute solution will behave as a polyelectrolyte Fuoss (1948).

For simple electrolytes it is customary to plot the equivalent conductivity against the square root of the concentration. At low concentrations a linear plot which conforms to the Onsager theory is generally obtained. When a similar plot is made for a polyelectrolyte a linear plot is not obtained, but instead, the equivalent conductivity generally shows a very sharp nonlinear increase with decreasing polyelectrolyte concentration. This type of behavior has been qualitatively ascribed to an elongation of the polyion with dilution and a concomitant increase in the number of free counterions, thus leading to an increase in the number of conducting species in solution Nelson (1971).

2.5. Applications of gum arabic.

Exudate gums are used in a number of applications, mainly situated in the food area. However, there are also considerable non-food applications.

2.5.1. Food applications.

The use of gum arabic in foods has to be in accordance with the FDA Code of federal regulations Table (2.7). Gum arabic is mainly used in the confectionery industry, where it is incorporated in a wide range of products. It has a long

tradition of use in wine gums, where it produces a clarity that is higher than can be obtained with other hydrocolloids (Williams *et al*., 2000). Furthermore, it prevents sucrose crystallization, provides a controlled flavor release, and slows down melting in the mouth, making the wine gum long lasting (Imeson 1992). It also provides the appropriate texture to these candies, which are easily deformed in the mouth but do not adhere to the teeth. In lower-calorie candy, gum arabic is used to compensate for the loss of texture, mouth feel, and body, resulting from the replacement of sugars by artificial sweeteners. It is also used in chewing gum as a coating agent and as a pigment stabilizer. In aerated confectionery products, such as marshmallows, nougats, and meringues, gum arabic acts as a whipping and stabilizing agent. It is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the product. In jelly products, it is used to provide a fibrous, Fruit-like texture (Shigeo *et al*., 1989). Gum arabic glazes are used as coatings for nuts, dragees, and others. Gum arabic is widely used as an emulsifier in the manufacture of soft drinks. Due to its stability in acid conditions and its high solubility, gum arabic is well suited for use in citrus and cola flavor oil emulsions. High levels of gum are used to ensure a complete coverage of the interface and to prevent flocculation and coalescence of oil droplets. Normally, a weighting agent is added to increase the oil-phase density, inhibiting destabilization due to creaming. Gum Arabic can also form a stable cloud in the drink, imitating the effect of added fruit pulps and juices. In beer, it is used as a foaming agent and to assist lacing (Tiss *et al*., 2001).Gum arabic is used increasingly as a source of soluble fiber in low-calorie and dietetic beverages (Phillips 1998). In powdered beverage mixes, gum arabic is added to produce the same opacity, appearance, mouth feel, and palatability as natural fruit juices. In microencapsulation, liquid, solid or gaseous substances are coated with a protective layer to prevent chemical deterioration and the loss of volatile

compounds. It is a useful technique to convert liquid food flavors to allowable powders that can be used in dry food products (McNamee *et al*., 1998). Gum arabic is an effective encapsulation agent because of its high water solubility, low viscosity, and emulsification properties and is used in soups and dessert mixes. Gum arabic is also used to prevent gelation in canned gravy based pet foods, as it inhibits the extraction of proteins from the meat into the gravy (Glicksman *et al*., 1975).

2.5.2. Non- food applications.

Gum arabic was extensively used in the pharmaceutical industry, but now is replaced by celluloses and modified starches in many applications. It is still used as a suspending agent, emulsifier, adhesive, and binder in tableting and in demulcent syrups. In cosmetics, gum arabic functions as a stabilizer in lotions and protective creams, where it increases viscosity, imparts spreading properties, and provides a protective coating and a smooth feel. It is used as an adhesive agent in blusher and as a foam stabilizer in liquid soaps (Whistler 1993).Gum arabic is also used in the preparation of etching and plating solutions in the lithography industry (FAO1995). It is used as a dispersant in paints and insecticidal acaricidal emulsions, respectively keeping the pigments and active components uniformly distributed throughout the product. In the textile industry, it is used as a thickening agent in printing pastes for the coloration of knitted Cellulose fabrics other applications are ink and pigment manufacture, ceramics, and polishes (Fuyama, *et al*., 1981).

Table (2.7): Maximum usage levels (%) of gum arabic permitted in accordance with the FDA Code of federal Regulations (Verbeken *et al*., 2003).

Food (as served)	Percentage	Function
Beverages and beverage bases	2.0	Emulsifier and emulsifier salt, flavoring agent/adjuvant formulation aid, stabilizer/thickener
Chewing gum	5.6	Flavoring agent/adjuvant, humectant, surface-finishing agent
Confections and frostings	12.4	Formulation aid, stabilizer/thickener, surface-finishing agent
Dairy products analogues	1.3	Formulation aid, stabilizer/thickener
Fats and oils	1.5	Formulation aid, stabilizer/thickener
Gelatins, puddings, and fillings	2.5	Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener
Hard candy and cough drops	46.5	Flavoring agent/adjuvant, formulation aid
Nuts and nut products	8.3	Formulation aid, surface-finishing agent
Quiescently frozen confectionery	6.0	Formulation aid, stabilizer/thickener
Snack foods	4.0	Emulsifier, emulsifier salt, formulation aid
Soft candy	85.0	Emulsifier, emulsifier salt, firming agent, flavoring agent/adjuvant, formulation aid, humectant, stabilizer/thickener, surface-finishing agent
Other food categories	1.0	Emulsifier,emulsifier, salt, flavoring agent/adjuvant, formulationaid,stabilizer/thickener, surface-finishing agent, texturizer

2.6. Rheological properties of *Acacia senegal* gum, glucuronic acid and glucuronates

Material scientists have investigated the flow and strain properties of materials since the 17th century. The term rheology was first used by physics and chemistry by E.C Bingham and M. Reiner on 29 April 1929 when the American Society of Rheoloy was founded in Columbus, Ohio.

All materials exhibit a response to an external force between the two extremities of ideal behavior: elastic solid and viscous liquid. An elastic solid is described by Hooke's law, and an ideal viscous liquid obeys the Newton's law. However, most food behaves as viscoelastic material; depending on the stress applied and the time scale, a solid body may have liquid phase properties and a liquid material can show solid body properties. The viscoelastic behavior of food has been widely studied in rheometers of sheared samples (tangential force), whereas the rheological parameters of tension or compression (normal force) are being increasingly used to characterize the texture of food products. Furthermore, it is possible to characterize the product to low or high deformations irrespective of the type force applied. Therefore, the rheology is extremely important for the food industry, mainly in the development of product formulations in which there is total or partial replacement of sugar, as the case with preserves and jellies with low soluble solids content. An effective approach to the technological problems due to this substitution, such as the loss of sweet taste, desired viscous texture and increased water activity, requires a deep understanding of the functionality of ingredients in product development, quality control studies of shelf-life and determination of the texture of the food (Pereira *et al*.;2013).

Rheological properties have been considered to be important analytical tools to provide fundamental in sights on the structural organization of food and play an important role in heat transfer. Foods are liquid, solid and semi-solid. Some foods especially starches and proteins undergo changes or modifications during processing resulting in a viscous dispersion, solutions or gel depending on temperature and concentration. Generally, fluids with suspended particles have a certain structure, which is sensitive to shear. Hence, steady-shear viscosimetry is not ideally suited if one wants to probe the rheological characteristics of an unperturbed dispersion. In oscillatory rheometry, the specimen is subjected to a very small oscillatory stress, such that its structure remains intact. The small-amplitude oscillatory (dynamic) tests (SAOS) have been commonly used to characterize the viscoelastic behavior of foods and allow researchers to relate dynamic rheological parameters to the samples molecular structure and glass transition temperatures). Furthermore, an empirical relationship between complex viscosity and steady shear viscosity known as the Cox–Merz rule (Cox & Merz, 1958) allows estimating steady shear viscosity for cases where its direct measurement is rather difficult. In addition, relationship among various rheological parameters is of interest to predict one from another for process industries and food product development. Though originally developed for synthetic polymers, the Cox–Merz rule and/or its modified forms apply to many liquid and semi-solid foods (Ahmed *et al*., 2006).

Rheological measurements are quite relevant in the food industry as a tool for physical characterization of raw material prior to processing, for intermediate products during manufacturing, and for finished foods. There are several approaches to conduct these rheological characterizations, and the selected

technique pretty much depends on the specific product and the functional characteristics in need to be analyzed (Munizaga *et al*; 2005).

Rheoloical parameters are mechanical properties. They include physical properties of liquids and solids which describe strain and flow behavior (temporal variation of strain). Strain is observed in all materials and substances when exerting external forces.

- **Rheometry** describes measuring methods and devices used to determine rheological properties. If an external force is exerted on a body, its particles will be displaced relative to each other. This displacement of particles is known as strain .Type and extent of strain are characteristic properties of a body.
- **Ideally elastic bodies** undergo elastic strain if external anisotropic forces are exerted on them. The energy needed for this strain is stored and effects spontaneous full recovery of the original form if the external force ceases to act.
- **Ideally viscous bodies** undergo an irreversible strain if external anisotropic forces (e.g. gravitational force) are exerted on them. The input energy is transformed. This increasing viscous strain is known as **flowing**. There are only few fluids with practical importance which show (almost) ideal viscous behavior. Most materials are neither ideally viscous nor ideally plastic. They rather exhibit different behavior and are thus called viscoelastic materials.

The simplest model to illustrate rheological properties is the **parallel plate model** as presented in Figure (2.8). The top plate, which has a surface area A [m^2], is moved by a force F [N = kgm/s^2] at a speed v [m/s]. The bottom plate remains at rests. The distance between the plates, to which the material adheres is described

by h [m]. If the thinnest elements of the liquid be displaced between the plates, this **laminar flow** is of fundamental importance for rheological investigations. Turbulent flows increase the flow resistance, thus show false rheological properties.

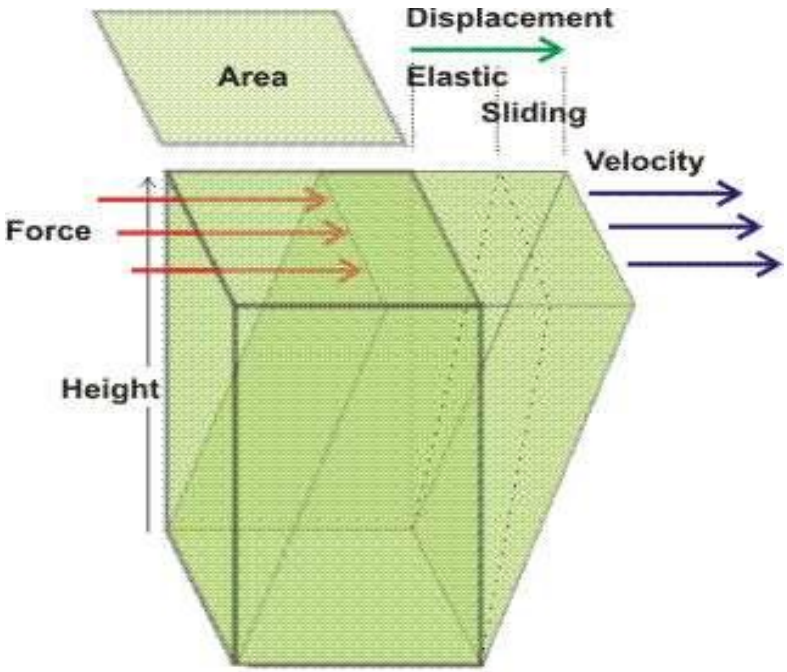


Figure (2.8): Parallel plate model

Shear rate $\dot{\gamma} = v / h$ in s^{-1} …….. (2.09)

Shear stress $\tau = F / A$ in Pa..... (2.10)

Shear viscosity $\eta = \tau / \dot{\gamma}$ in Pas……. (2.11)

Strain $\gamma = dx / h$ dimensionless..... (2.12)

In addition to the expression $\dot{\gamma}$, the symbol D is also used for the shear rate.

Shear tests are usually conducted using rotational viscometer. In contrast to the parallel plate model, the moved surface performs a rotary movement.

2.6.1. Definitions of some rheological terminology

2.6.1.1. Rheology

Rheology concern flow and deformation of materials in particular, to their behavior in the transient area between solids and fluids. Moreover, rheology attempts to define relationship between the stress acting on a given material and the resulting deformation or flow that takes place.

2.6.1.2 Shear stress

Force F acting on area A to effect a movement in the liquid element between the two plates. The velocity of the movement at a given force is controlled by the internal forces of the material.

$$shear\ stress\ \tau = \frac{Force\ F}{Area\ A} = \frac{N(Newton)}{m2} = Pa \qquad \text{..... (2.13)}$$

100 Pa = 1 mbar = 1 hPa.

Old unit: dyne / cm^2 = 0.1 Pa.

2.6.1.3. Shear rate

By applying shear stress a laminar shear flow is generated between the two plates. The uppermost layer moves at the maximum velocity v_{max}, where the lowermost layer remains at rests. The shear rate is defined as:

$$shear\ rate\ \dot{\gamma} = \frac{\mathrm{d\ v}}{d\ h}$$ ..... (2.14)

Where dv is velocity differential between adjacent velocity layers. d*h* is thickness differential of the flow layers.

In a laminar flow the velocity differential between adjacent layers of like thickness is constant (dv = constant, d*h* = constant), the differential can then be approximated as follows:

$$shear\ rate\ \dot{\gamma} = \frac{\text{velocity v}}{distance\ h} = \frac{m/s}{m} = s - 1$$ ..... (2.15)

Table (2.8) show shear rate ranges which applied to each specific food in industry.

Table :(2.8) Shear Rate Ranges

Process	**Shear rates in s^{-1}**	**Example of use**
Sedimentation of fine particles in suspensions	10^{-6} ---- 10^{-4}	Paints, lacquers, pharmaceutical solutions
Flowing due to surface characteristics	10^{-4} ---- 10^{-1}	Paints, printing inks
Dripping under the effect of the gravitational force	10^{-2} ---- 10^{1}	Paints, coatings
Extruding	10^{0} ---- 10^{2}	polymers
Chewing / swallowing	10^{1} ---- 10^{2}	food
Spreading butter on a slice of bread	10 ----- 50	food
Mixing, agitating	10^{1} ---- 10^{3}	Substances in process engineering
brushing	10^{2} ---- 10^{4}	Paints, lacquers, pastes
Spraying, spreading	10^{3} ---- 10^{6}	Paints, lacquers, coatings
Rubbing in	10^{4} ---- 10^{5}	Creams, lotions
High-speed coating	10^{5} ----10^{6}	Paper coatings
Lubricant of machine parts	10^{3} ---- 10^{7}	Mineral oils, greases

2.6.1.4. Dynamic viscosity

Viscosity describes the toughness of a material.

$$\text{Dynamic viscosity } \eta = \frac{\text{shear stress } \tau}{shear\ rate\ \dot{\gamma}}\ in\ \frac{pa}{s-1} = Pas$$

…… .(2.16)

The unit Pas (or mPas) is used for the viscosity.

1 Pas = 1000 mPas.

Old unit: 1 P (Poise) = 100 cP (Centipoises) = 100 mPas (Millipascal second).

2.6.1.5. Kinematic viscosity

If ideally viscous materials are tested using a capillary viscometer, such as an Ubbelohde viscometer, the kinematic viscosity ν is determined, not the dynamic viscosity η.The kinematic viscosity is related to the dynamic viscosity through the density of the material.

$$\text{Kinematic viscosity } \nu = \frac{\text{dynamic viscosity } \eta}{density\ \rho}\ in\ \frac{mm2}{S}$$

..... (2.17)

Old unit: cSt (Centistokes) = mm^2 /S.

2.6.2. Rheological Classifications

2.6.2.1. Newtonian Fluids

The use of flow models is the most basic form of data processing in rheology, and indeed, most flow models more properly belong in viscometry because the elastic behavior of the material is not even considered. The emphasis of such models is prediction of the flow behavior over a range of shear rates or shear stresses. Newton's Postulate from the "Principia" is the simplest model for flow and also one of the earliest dating from 1687. This postulate which describes an ideal viscous liquid is based on a linear relationship between stress and shear rate where the proportionality constant is viscosity.

Stress (σ) = Viscosity [η] · Strain Rate [$d\dot{\gamma}/dt$] ……… (2.18)

Unfortunately, very few materials are Newtonian. Only simple fluids with small non-interacting molecular components such as oils, water, and sugar solutions behave this way.

2.6.2.2. Non -Newtonian Fluids

Most fluids are non-Newtonian, non-linear models are needed to describe the change in viscosity with shear rate or shear stress. The simplest of these non-linear models is a Power Law, where viscosity [either Newtonian or apparent] is replaced by a consistency coefficient [K]. Furthermore, the index of shear rate in the Newtonian model can be considered to be 1, but the Power Law, as its name implies, has a flow behavior index n (where n = 1), which designates just how non-linear the curve is. There are two types of Power Law; the first describes pseudoplastic [shear thinning] materials which dominate the field of rheology. Most materials are shear thinning because the input shear energy tends to align an

isotropic molecules or particles and disaggregate any large clumps of particles, thereby reducing the overall hydrodynamic drag, which in turn reduces the dissipation of energy in the fluid and the viscosity. On the other hand, some materials show an increase in viscosity as shear rate or stress increases. This [shear thickening] is relatively unusual, occurring mainly in dispersions of particles where the volume fraction is relatively high. Shear thickening can sometimes even result in a volumetric expansion as the dispersed solids are forced together, squeezing out the suspending phase. The Power Law can model both situations by simply having a flow behavior index either greater than one [shear thickening] or less than one [shear thinning]. Figure (2.9) give an explanation of some rheological models.

PSEUDOPLASTIC $\sigma = K \dot{\gamma}^{n}$ (n) <1 …… …… (2.19)

DILATANT $\sigma = K \dot{\gamma}^{n}$ (n) >1 …… (2. 20)

An additional level of complexity is introduced if the fluid rheogram [plot of shear stress vs. shear rate] has a non-zero intercept. If the line crosses the shear stress axis at a fixed value, then all stresses below that value are assumed to result in a zero flow situation. The value of the stress where this occurs is called the "Yield" stress and for many years this stress was thought to be a constant for a specific material. With the advent of more sensitive viscometers, however, workers found that the yield stress decreased once lower shear rates could successfully be set. Eventually, controlled stress rheometers showed that the yield stress was an apparent phenomenon whose magnitude depends on the measurement technique used. The majority of materials are thought to exhibit a high but finite viscosity known as the zero shear viscosity, rather than an infinite viscosity below a certain yield stress. Three models include a yield stress term. These models range in

complexity from a linear model with an added yield stress [Bingham] to a linear model taken as the square root [Casson] to a Power Law with an added yield stress [Herschel-Bulkley] (Barnes *et al*., 1993).

BINGHAM $\sigma = \sigma_y + \eta_p \dot{\gamma}$ ……(2.21)

CASSON $\sigma^{1/2} = \sigma^{1/2} + \eta^{1/2} \dot{\gamma} c^{1/2}$ …….. (2.22)

HERSCHEL-BULKLEY $\sigma = \sigma y + K \dot{\gamma}^n$ …….. (2.23)

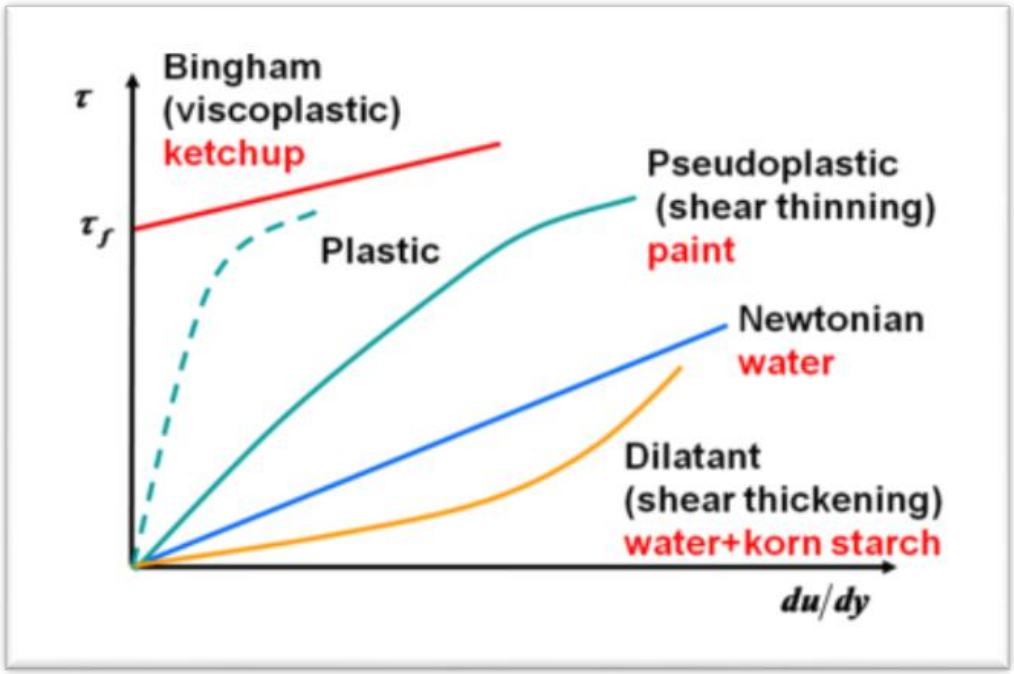


Figure (2.9): Rheological models as function of shear rate vs. shear stress

2.6.3. Dynamic oscillatory testing

Dynamic test is performed applying a small sinusoidal strain (stress) and measuring the resulting stress (strain). These small-amplitude oscillatory tests are

commonly performed in shear and hence the abbreviation SAOS, for small-amplitude oscillatory shear, is commonly used to represent dynamic viscoelastic tests. Though not yet very popular, the dynamic tests are also performed in compression and tensile mode and in large amplitude shear mode (at strains in excess of 100%). It is important to emphasize that the strains (and stresses) used in SAOS tests are very small, often in the order of 1 to 3 or 5%. This is to assure that the material response is in the linear range; the range within which the stress is proportional to the applied strain, and the theory described below is applicable. The stress response of a linear viscoelastic material to a sinusoidal strain input is given as:

$$\sigma(t) = \gamma'_o G'(\omega) \sin(\omega t) + \gamma'_o G''(\omega) \cos(\omega t) \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.24)$$

The frequency dependent functions $G'(\omega)$ and $G''(\omega)$ are shear elastic (storage) modulus and shear elastic (loss) modulus, respectively. G' is a measure of the energy stored and subsequently released per cycle of deformation per unit volume and it is the property that relates to the molecular events of elastic nature. G'' is a measure of the energy dissipated as heat per cycle of deformation per unit volume. G'' is the property that relates to the molecular events of viscous nature. Another commonly used dynamic viscoelastic property, the loss tangent tan tan $\delta(\omega t) = G''/G'$ denotes relative effects of viscous and elastic components in a viscoelastic behavior. Sometimes the complex modulus $G^* = \sqrt{G'^2 + G''^2}$ is also used to describe dynamic test data. Similarly, complex viscosity (η^*) can also be defined in terms of real (η') and imaginary (η'') parts of viscosity. The quantities G', G'', and η', η'' enable the rheological characterization of a viscoelastic material on the basis of an SAOS test (Gunasekaran, 2000).

Ideal elastic behaviour is expressed as $\delta = 0^{o}$, or tan $\delta = 0$, because G' completely dominates G'', while ideal viscous behavior is expressed as $\delta = 90^{o}$, or tan $\delta = \infty$, because G'' completely dominates G'. When viscous and elastic behavior exactly balance (i.e. when $G' = G''$), then tan $\delta = 1$ or $\delta = 45^{o}$, and a crossover of G' and G'' values was noticed at a certain frequency. The crossover frequency provides a good indication of the viscoelastic behaviour of the material. Materials with a lower crossover value generally implied a higher elastic contribution to their viscoelastic properties. A typical gel behaviour is indicated by a higher G' than G'' throughout the frequency range tested (Ramachandran, *et al.*, 1999).

2.6.4. Rheomertry

Rheometers are devices used to determine viscosity and other rheological properties of materials. Relationships between shear stress and shear rate are derived from physical values of system configurations, pressures, flow rates, and other applied conditions. There are two common methods used for rheometric measurements on fluid systems: capillary (viscometers) and rotational rheometers. Viscometers are devices used principally for the measurement of viscosity, while rheometers are devices used for the measurement of rheological properties of materials, typically fluids and melts (Tuhumury *et al*., 2014).

2.6.4.1. Rotational Viscometry

For fluids, the primary mode for rheological measurement in the food industry is rotational viscometry, providing rapid and fundamental information. Rotational viscometry involves a known test fixture (geometrical shape) in contact with a sample, and through some mechanical, rotational means, the fluid is sheared by the fixture. Primary assumptions are made for the development of constitutive equations (relationships between shear stress and rate).

Rotational rheometers may operate in two modes: steady shear or oscillatory; two test fixtures most often used in steady shear rotational viscometry are concentric cylinder and the cone and plate (Torres *et al*., 2014).

2.6.4.1a. Concentric Cylinders

This rheological attachment consists of a cylindrical fixture shape, commonly called a **bob** with radius *R*b, suspended from a **torque** (*M*) measuring device that is immersed in a sample fluid contained in a slightly larger cylinder, referred to as the **cup** with radius *R*c. Torque is an action that generates rotation about an axis and is the product of a force and the perpendicular distance (*r*), called the **moment arm**, to the **axis of rotation**. The principals involved can be described relative to changing tires on a car. To loosen the lugnuts, a larger tire iron is often required. Essentially, this longer tool increases the moment arm, resulting in a greater torque about the lugnut. Even though you are still applying the same force on the iron, the longer device provides greater torque.

One of the most common rheological devices found in the food industry is the **Brookfield viscometer** as show in Figure (2.10)**.** This affordable apparatus uses a spring as a torque sensor. The operator selects a rotational speed (rpm) of the bob, attached to the spring. As the bob moves through the sample fluid, the viscosity impedes free rotation, causing the spring to wind. The degree of spring windup is a direct reflection of the torque magnitude (*M*), used to determine a shear stress at the bob surface.

2.6.4.1b. Cone and Plate

Another popular system for rotational measurement is the **cone** and **plate** configuration presented in Figure (2.11). Its special design permits the shear stress and shear rate to remain constant for any location of sample in the fluid gap. Test

quality is best when the cone angle (θ) is small, and large errors may be encountered when the gap is improperly set or not well maintained. A primary advantage of the cone and plate test fixture is that shear stress and rate are independent of position – constant throughout the sample. The parallel plate geometry can be considered a simplified version of the cone and plate, having an angle of 0^0. The test fluid is constrained in the narrow gap between the two surfaces. Cone and plate and parallel plate measurement tools are most often used for highly viscous pastes, gels, and concentrated suspensions Daubert (2010)

Figure (2.10): Brookfield viscometer.

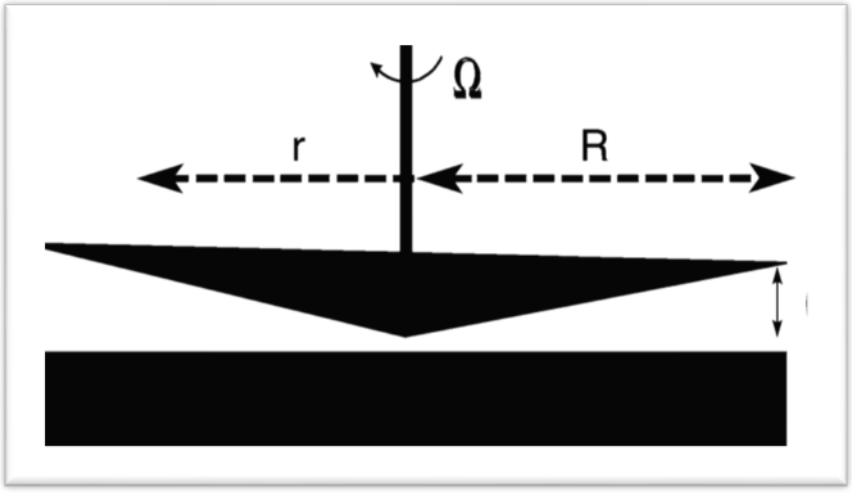


Figure (2.11): Cone and plate configuration

2.6.5. Applications of Rheology

Rheology has applications in materials science engineering, geophysics, physiology, human biology and pharmaceutics. Materials science is utilized in the production of many industrially important substances, such as cement, paint, and chocolate, which have complex flow characteristics. In addition, plasticity theory has been similarly important for the design of metal forming processes. The science of rheology and the characterization of viscoelastic properties in the production and use of polymeric materials have been critical for the production of many products for use in both the industrial and military sectors. Study of flow properties of liquids is important for pharmacists working in the manufacture of several dosage forms, such as simple liquids, ointments, creams, pastes etc. The flow behavior of liquids under applied stress is of great relevance in the field of pharmacy. Flow properties are used as important quality control tools to maintain the superiority of the product and reduce batch to batch variations (Yaseen *et al*., 2005).

Rheology is important in illustrating practical problems in the processing and use of rubbers, plastics, and fibers. Polymers constitute the basic materials of the rubber and plastic industries and are of vital importance to the textile, petroleum, automobile, paper, and pharmaceutical industries. Their viscoelastic properties determine the mechanical performance of the final products of these industries, and also the success of processing methods at intermediate stages of production. In viscoelastic materials, such as most polymers and plastics, the presence of liquid-like behavior depends on the properties of varies with rate of applied load, i.e., how quickly a force is applied. The silicone toy 'Silly_Putty' behaves quite differently depending on the time rate of applying a force. Pull on it slowly and it exhibits continuous flow, similar to that evidenced which have a highly viscous liquid.

Physiology investigations include the study of many bodily fluids that have complex structure and composition, and thus exhibit a wide range of viscoelastic flow characteristics. In particular there is a specialist study of blood flow called hemorheology. Which study flow properties of blood and its elements (plasma and formed elements, including red blood cells, white blood cells and platelets). Blood viscosity is determined by plasma viscosity, hematocrit (volume fraction of red blood cell, which constitutes 99.9% of the cellular elements) and mechanical behaviour of red blood cells. Therefore, red blood cell mechanics is the major determinant of flow properties of blood (Diaz *et al*., 2003).

Food rheology is important in the manufacture and processing of food products. Thickening agents, or thickeners, are substances, when added to an aqueous mixture; increase its viscosity without substantially modifying its other properties, such as taste. They provide body, increase stability, and improve suspension of added ingredients (Mckenna *et al*., 2006).

2.7. Emulsification properties of *Acacia senegal* gum, glucuronic acid and glucuronates

Food hydrocolloids are high molecular weight hydrophilic biopolymers used as functional ingredients in the food industry for the control of microstructure, texture, flavor and shelf life. The term ‹hydrocolloid‘embraces all the poly saccharides that are extracted from plants, seaweeds and microbial sources, as well as gums derived from plant exudates, and modified biopolymers made by the chemical or enzymatic treatment of starch or cellulose. In addition due to its poly disperse and highly hydrophilic character, the unique protein gelatin has become accepted as an exceptional member of this polysaccharide club, but other food proteins, like casein and gluten, are traditionally not classified as hydrocolloids even though they certainly do exhibit functional properties that overlap considerably with those of the food polysaccharide.

Food hydrocolloids are multi-phase food system containing particles or other structures with characteristic spatial dimensions in colloidal size range. The term ‹colloid‘can be applied to particulate dispersions, foams, gels and emulsions (oil in water, water in oil). Therefore, most manufactured food stuffs can be classified as food colloids, and many contain hydrocolloid ingredients added by the manufacturer for control of stability and rheological properties. An important class of food colloids is oil in water emulsions (Dickinson, 2003).

One of the key functional roles of food hydrocolloids is in the preparation of emulsions and in the control of emulsion shelf-life. Emulsions are one of the most significant classes of food colloids; they are unstable and tend to destabilize by flocculation, coalescence, creaming, phase inversion and Ostwald ripening. Figure (2.12), show diagram of most common instability mechanisms of emulsion.

Polysaccharides are often added as stabilizing and thickening agents, in order to alter the rheological characteristics or stabilize the emulsions (Makri *et al*., 2006).

The most widely used polysaccharide emulsifiers in food applications are gum arabic (*Acacia senegal*), modified starches, modified celluloses, some kinds of pectin, and some galactomannans (Dickinson; 2008).

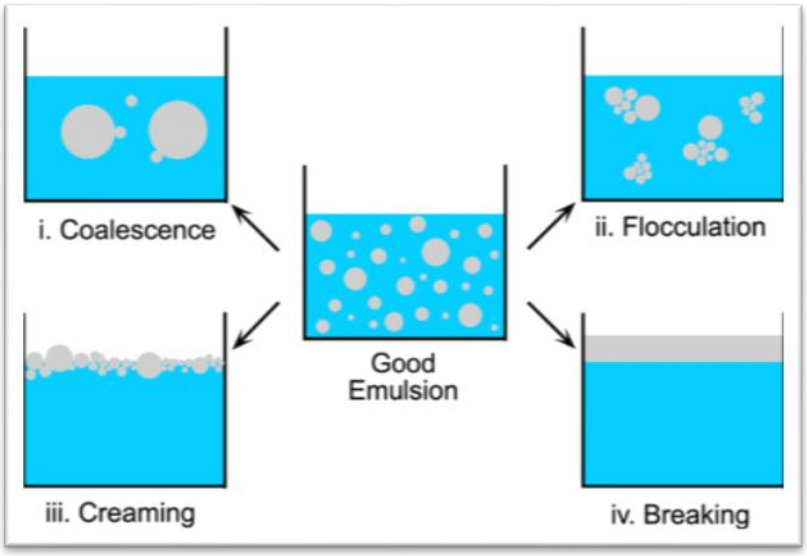


Figure (2.12): diagram of most common instability mechanisms

2.7.1. 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 presence of emulsifying agent. The 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).

2.7.2. Classification of emulsion

Emulsions can be classified according to the relative spatial distribution of the different phases in to three types as shown in Figure (2.13):

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

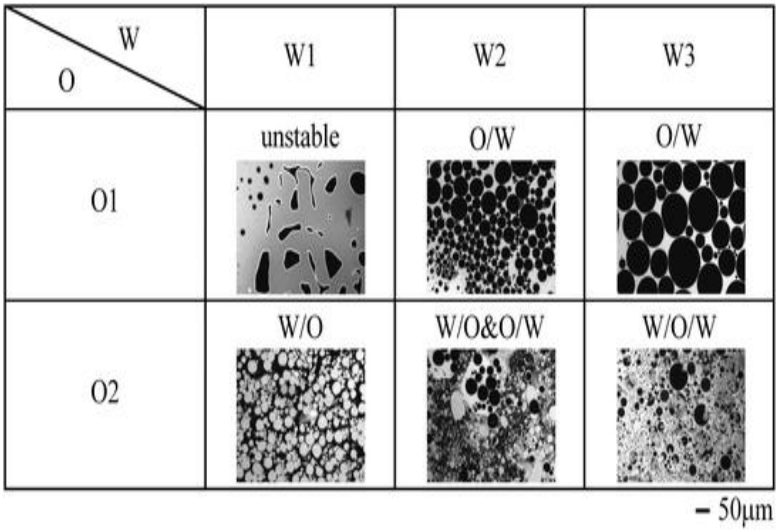


Figure (2.13): Types of emulsions

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). Macro emulsions are inherently thermodynamically unstable systems because the contact between oil and water molecules is unfavorable, and so they will always breakdown over time.

2.7.3. Processes involved in making of emulsions

To form a fine emulsion, large deformable drops must be broken down by the vigorous application of mechanical energy. In food processing this can be traditionally achieved using a high-speed mixer, a colloid mill, or a high-pressure valve homogenizer. Thermodynamically speaking, the process is extremely inefficient, with most of the power being dissipated as heat. Emulsification involves the sudden creation of a large amount of new liquid interface. Thermodynamics tells us that, in order to increase the oil–water surface area by an amount ΔA, the required work (free energy change) is $\Delta G = \gamma \Delta A$, where γ is the interfacial tension. If we want to make an oil-in-water emulsion of 10 vol. % oil containing uniform droplets of radius 1 μm using an emulsifier which reduces the interfacial tension to γ 5 mN m^{-1}. From thermodynamics, we can estimate that the theoretical work associated with making the new interface is ΔG 10^3 J m^{-3}. But in practice the actual amount of work required to make such an emulsion is of the order of 10^6 J m^{-3}, i.e., a thousand times larger. The reason for this gross discrepancy is that small droplets have highly curved interfaces, and the breaking of larger droplets into smaller ones requires the rapid application of a disruptive

force to overcome the interfacial forces holding the larger droplet together. To disrupt a droplet of radius a requires an external pressure gradient of magnitude $\Delta p/a = 2\gamma/a^2$, where Δp is the Laplace pressure. This implies a pressure gradient of the order of 10^{10} Pa m^{-1} (i.e. 1 kbar cm^{-1}). During homogenization, the fluctuating stress differences needed to produce such a high local pressure gradient are generated from the intense laminar flow (shear and extensional deformations) or inertial effects (turbulence and cavitation) (Dickinson, 2008).

2.7.4. Emulsifier (emulsifying agent)

The preparation of emulsions that are kinetically stable over a time period requires the incorporation of substances known as stabilizers or emulsifying agent (Dickinson, 1992). The main role of the emulsifier is to stabilize emulsions by adsorbing at the particle interface. The hydrophilic portions align themselves within the lipid phase, while the lipophilic portions align in the water phase as presented in Figure (2.14). Dicknison and Sainsby (1988) distinguished between emulsifier and stabilizer in food system. Emulsifier is defined as a single chemical or mixture of components having the capacity for promotion emulsion formation and short term stabilization by interfacial action while stabilizer is defined as a single chemical or mixture of components which can offer long term stability on emulsion, possibly by mechanism involving adsorption.

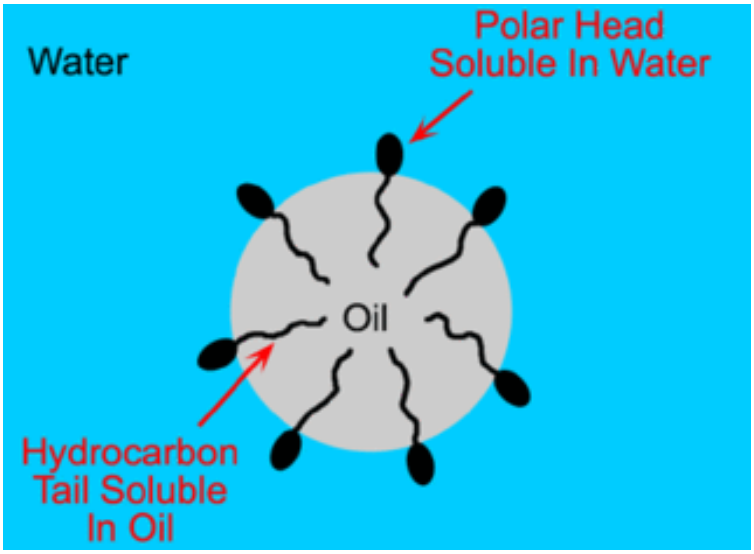


Figure (2.14): Schematic diagram of emulsifying agent

An effective emulsifier is therefore one that (i) rapidly reduces the interfacial tension at the freshly formed oil–water interface, (ii) binds strongly to the interface once adsorbed, and (iii) protects the newly formed droplets against flocculation or coalescence. This protection against immediate recoalescence occurs in the first place *via* dynamic surface tension effects (the Gibbs–Marangoni mechanism) and later *via* repulsive colloidal interactions (electrostatic and steric stabilization mechanisms).With polymeric emulsifiers at relatively low concentrations, the kinetics of emulsifier adsorption is commonly the rate-determining factor. In many food situations, the kinetics of adsorption and the processes of interface stabilization are complicated by the fact that the emulsifying species are poly disperse in size and in chemical composition. During emulsification with protein as the main emulsifier, the presence of even a small quantity of rapidly adsorbing surfactant can facilitate substantial reduction in the mean droplet size. A rather higher concentration of a miscible cosolute (e.g., 10–20% ethanol), whose presence lowers the surface tension of the aqueous phase, can play a similar role. Due to the Gibbs–Marangoni mechanism, not all the fresh oil–water interface has

to be fully saturated with emulsifier for the individual droplets to be protected against recoalescence. It should be noted, however, that the Gibbs–Marangoni effect only operates if the emulsifying agent is present in the continuous phase (Dickinson, 2008).

A number of food components exhibit these characteristics and can be used as emulsifier (e.g.; gums, lecithin, proteins, modified starches, phospholipids, etc.). However, these emulsifiers vary considerably in their molecular structure, which influences their ability to form and stabilize emulsions, as well as to withstand environmental stresses, such as variations in ionic strength, pH, and temperature. Emulsifier performance is one of the most important factors determining the formation and stability of emulsions and several special emulsifier characteristics have to be considered in selecting emulsifiers for producing emulsions (Zhang; 2011).

It is generally important that emulsion droplets are made as small as possible in order to minimize aggregating effects. A convenient way to evaluate the relative effectiveness of an emulsifier under controlled hydrodynamic conditions is to determine the bulk emulsifier concentration required to produce the minimum mean droplet size (maximum surface area per unit volume of oil).

2.7.5. Emulsification properties of gum arabic.

Gum arabic is highly heterogeneous polysaccharide, a natural exudate of *Acacia senegal* trees which is widely used in the beverage industry. It is a complex proteoglycan acid salt (mainly Ca, K, Mg and Na) of high molecular weight (average 4×10^5). It is composed of D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and its 4-Omethyl ether. It also has a proteinous component, which is an integral part of the gum structure, composed by three components (Elzain *et*

al; 2012). Two of them do not adsorb at the interface, the third one, which is 10% of total gum with a high protein content (50%), which does adsorb at the interface is responsible for the emulsifying properties of the gum (Islam *et al*; 1997). The most accepted model for the emulsifying fraction of the arabic gum is the ‘‘wattle blossom’’ (Osman *et al*; 1993). There are other models, which involve electrostatic contribution as well as steric forces, have also been proposed (Islam *et al*.; 1997).

The gum from *Acacia senegal* has a functional ability to act as emulsifier that stabilizes oil-in-water emulsion. Its arabino galacto protein (AGP) component is responsible for its emulsifying properties, including the ability to form stable emulsion over a wide pH range and in the presence of electrolytes. It is now known that the protein-rich high molecular 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. Therefore, protein moiety had effects on emulsifying behavior of gum Arabic and the best emulsion capacity and stability is found in gums with highest nitrogen content. The emulsifying samples properties of gum arabic, however, are directly influenced by the botanical type, the nature of the growing soils and the climate (Sabah El-Kheir *et al*; 2008).

Previous studies have also shown that the stability of oil-in-water (O/W) emulsions depends on both the type and concentration of ingredients contained in the emulsion as well as processing and storage conditions. Concentrated emulsions are a unique class of O/W emulsion in that they can be consumed in highly diluted form (low viscosity fluids such as milk and fruit juice beverages) or in their original concentrated form (such as in creams, margarine or butter).Thus, the

emulsion must have a significant degree of stability in both the concentrated and diluted forms (Achouri *et al*; 2012).

The level of surface activity is rather low in comparison with typical food protein emulsifiers. Therefore, to generate stable sub-micron-sized droplets, it is necessary to use a rather high gum-to-oil weight ratio, approximately 1:1, as compared with 1:10 for equivalent protein-stabilized emulsions. Once formed by adsorption on to the macroscopic oil–water interface, there is little effect on the high surface shear viscosity of the gum film by large dilutions of the aqueous sub-phase. The film viscoelasticity is, therefore, maintained even when a major proportion of the hydrocolloid has been removed from the aqueous phase in contact with the adsorbed layer. Thus, only a small proportion of the gum used in emulsion preparation is actually involved in the stabilization process (Dickinson; 2003).

On the other hand, there does appear to be a good correlation between emulsion stability and gum arabic average molecular weight. It was observed that the 10% fraction of gum arabic sample corresponding to the highest molecular weight (38%N) as separated by gell permeation chromatography, could produce a more stable emulsion than the remaining 90% fraction (35%N) was gradually reduced from 3.1×10^5 to 2.2×10^5 Da by controlled irradiative degradation, the resulting emulsion stability was correspondingly reduced. This trend is consistent with the formation of thicker and more effective steric stabilizing layers by adsorption of polymers of greater molecular mass (Dickinson; 2003).

2.7.6. Evaluation of emulsion stability

Emulsification stability was evaluated by the change in the particle size of emulsion after stored for 3 and 7 days. Emulsion stability index was taken as a

parameter to designate the grade of the gum sample. Therefore, gum samples which showed a change of 0.7 µm or less were classified as grade 1 (good emulsifier). A change between 0.7–0.85 µm was classified as grade 2 less stable emulsions. A change >0.85 µm were allocated grade 3 (poor emulsifier).The parameter which exerted the greatest influence on the final oil droplet size is the high pressure applied during homogenization. For a fixed emulsifying system (oil concentration and emulsifier) there is a maximum interfacial area which can be covered by the emulsifier, and to generate this area the certain amount of energy must be supplied (McClements;1999).

2.7.7. Applications of emulsion

Emulsions can be used in variety of fields:

2.7.7.1. In food

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

- Creams (foam) in espresso – coffee oil in water (brewed coffee), unstable emulsion
- 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 stearoyl lactylate
- 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).

2.7.7.2. In medicine

In pharmaceutics, hairstyling, personal hygiene, and cosmetics, emulsions are frequently used. These are usually oil and a 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 form 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, so when they encounter the lipids in the cell membrane or envelope of bacteria or viruses, they force 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 non-porous surfaces.

2.7.7.3. 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 cover only the surface of the fuel to achieve vapor mitigation (Silvestre *et al*.; 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1. Gum sample

Acacia senegal gum sample was collected from Blue Nile State (Elddamazin) during season 2009.

3.1.2. Purification of crude gum

Gum nodules were dried at room temperature, and then cleaned by hand to ensure they were relatively free from sand, dust and bark impurities then ground and kept in a labeled container for analysis.

3.2. Analytical methods

The following analytical methods were adopted in this study:

3.2.1. Determination of moisture content

Two grams of gum powder were accurately weighed. Porcelain crucible was well dried in an oven at 105^0C for 30 minute, cooled in a desiccator and weighed (W_1). About two grams of the sample were placed in the crucible and weighed accurately (W_2), heated for 5 hours at 105^0C, cooled in a desiccator and weighed again (W_3), (FAO, 1999) .The loss on drying was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W2-W3}{W2-W1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

Where:

W_1: weight of the empty crucible (g).

W_2: weight of crucible +sample (g) before drying.

W_3: weight of crucible +sample after drying (g).

3.2.2. Determination of ash content

Porcelain crucible was well dried by heated at 550^0c, cooled in a desicator and weighed (W_1) about two grams of gum sample was accurately weighed in a crucible (W_2), ignited at 550^0C in a heracus electronic muffle furnace until free from carbon, cooled in a desicator and weighed (W_3). Then the total ash% was calculated (Flindt, 2005).

$$\text{Ash (\%)} = \frac{W3-W1}{W2-W1} \times 100 \qquad \text{.. (3.2)}$$

Where:

W_1: weight of the empty crucible (g).

W_2: weight of crucible + sample (g).

W_3: weight of crucible +ash (g).

3.2.3. Determination of specific optical rotation

Optical rotation (observed) was determined for 2.0% w/w aqueous solutions (on a dry weight basis) using Automatic digital polar- meter (P3001 RS Germany) fitted with sodium lamp. The reading of the angled rotation of the solution was tacken at 20^0C. Correction for rotation of solvent was done (Idris *et al*. 1998). The optical rotation was calculated according to the relationship:

$$\text{Specific optical rotation } [\alpha]_D^T = \frac{\alpha \, x \, 100}{L \, x \, C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

Where:

α = Observed angle of rotation.

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

C = concentration in gm/100ml

T = Temperature.

3.2.4. Determination of intrinsic Viscosity

12 ml of arabic acid and arabate salts solutions (1% w/v in 1 M and 0.5 M NaCl) were transferred each into an Ubbelohde viscometer (SCHOTT instruments, type 530 03 Germany) and left for 30 minutes to reach the thermostat temperature, adjusted at 25^{o} C. Each gum solution was diluted several times and the flow time was measured for each concentration in triplicates. Finally the flow time for the pure solvent (1 M and 0.5 M NaCl) was measured. Determinations were made both with and without the presences of NaCl. Intrinsic viscosity was obtained by plotting reduce viscosity vs concentration (Flindt, 2005)

3.2.5. pH measurement

For 1% aqueous solution using a Microprocessor pH meter (combined pH or ORP electrode, USA) was calibrated with a standard solution of known pH. The pH measurement of the gum solution was read from the instrument (Yebeyen, 2009).

3.2.6. Determination of nitrogen and protein content

The percentage of nitrogen was determined according to the Kjeldahl method. The Kjeldahl technique is the method commonly used for protein analysis in food products. The procedure consists of three basic steps which require a number of reagents: The first step is digestion which carried out with concentrated sulfuric acid in the presence of an inorganic catalyst, which accelerates reduction of all organic nitrogen present into an ammonium salt. The second step is separation of the ammonium formed using distillation and the capture of the ammonium with a weak acid (boric acid). The third step is the quantification of the ammonium by titration with a strong acid (hydrochloric acid) (Rossi *et al*., 2004). The reactions involved in these steps can be shown as follows:

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

3.2.6.1. Method

A semi micro Kjeldahl apparatus was used to determine the percentage of the total nitrogen in each sample. Accurately weighed 0.2 gram of each gum sample (in triplicates) was transferred to Kjheldahl digestion flask together with Kjeldahl tablet (copper sulphate - potassium sulphate used as a catalyst). Then 4 ml. concentrated (nitrogen free, sulphuric acid) was added to the flask. The flask was then mounted on the digestion heating system which was previously set to 240^o C and capped with an aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish-green colour was observed which indicates the completion of the digestion. The flask was then allowed to attain room temperature. Its content was

quantitatively transferred to Kjeldahl distillation apparatus followed by addition of distilled water and 30% (w/v) sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 10 ml. of 2% boric acid until the volume reached 50ml. Back titration of the generated borate was then carried out versus, 0.01M, hydrochloric acid using methyl red as an indicator. The same procedure was repeated for blank sample (distilled water).

$$\text{Nitrogen (\%)} = \frac{(volume\ of\ titrant - volume\ of\ blank\ X\ 14.01\ XM\ X100}{weight\ of\ sample\ (grams)\ X1000} \quad \ldots. (3.\ 4)$$

Whereas:

M is the molarity of hydrochloric acid

The protein content was calculated using the nitrogen conversion factor of 6.6 as proposed by Anderson (1986).

Protein (%) = Nitrogen (%) × 6.6 ……. (3. 5)

3.2.7. Determination of acid equivalent weight and uronic acid content

The acid equivalent weights and uronic acid content of the gum samples were determined by titration. A cation exchange column packed with amberlite (IR 120H^+) resin; was washed with 2.0 M H_2SO_4, followed by distilled water until it was free from sulfate ion (the eluent was checked for the absent of a precipitate by the addition of barium sulfate solution), then 50ml. of 3% w/v gum sample was slowly passed down the column. The eluent and washing (~300ml.) were collected and titrated potentiometrically against 1M NaOH (Idris *et al*., 1998).

The equivalent weight of acid was calculated as:

$$\text{Acid equivalent} = \frac{weight\ of\ sample \times 1000}{volume\ of\ titrant \times molarity\ of\ alkali} \ldots\ldots\ldots\ldots\ldots (3.6)$$

.

$$\text{Uronic acid \%} = \frac{\textit{molar mass of uronic acid anhydride} \times 100}{\textit{acid equivalent weight}} \quad \ldots\ldots\ldots\ldots (3.7)$$

3.2.8. Determination of the number average molecular weight by Osmometric method.

12% stock solution from each sample was prepared. Dilutions of the stock solution in to twelve different concentrations arranged from 12% - 1% g/cm was done by adding of appropriate amount of distilled water. Then each concentration injected in to the Osmostat 050 colloid Osmometer (Genotech Germany) and osmotic pressure was read in mmHg.

3.2.9. Refractive index

(Pinch refracto-meter) was used to detect 1%gum solution at 25^{o}C (Renard, 2006).

3.2.10. Sugar composition of gum arabic

Complete acid hydrolysis of gum arabic yields four basic sugar constituents; D-glactose, L-arabinose, L-rhamnose and D-glucuronic acid (Sharma, 1981).

3.2.10.1. Sample preparation

Samples were hydrolyzed to liberate sugar residues. o.5 grams of gum was accurately weighed into tarred coppered test tubes, 10 ml. of 4% H_2SO_4 was added and incubated in water bath at 100^{o}C for 7 hours. The tubes were removed from water bath and allowed to cool to room temperature. 2 gram of barium carbonate

was added to each tube to neutralized hydrolysis products until the effervescence of CO_2 stops. The tubes were allowed to stand in shaker overnight, and finally filter.

3.2.10.2. Method

The purpose of analyzing the gum samples by HPLC was to determine the relative concentration of each sugar residue present in the sample, namely rhamnose (Rha), arabinose (Ara), galactose (Gal) and glucuronic acid (GlcA). Before analysis of the gum samples calibration curves of standard sugars were performed. HPLC column is flushed with the mobile phase (acetonitrile: water mixture 70: 30 v/v), at flow rate of 1.0 cm^3/ minute till a stable base line is obtained.The hydrolyses of gum sample, which is filtered in line, is injected inqwto a 20 micro liter loop of the injection valve of the HPLC system. The injection valve is turned to allow for eluting the hydrolyte through NH2p-504E amino column. Retention time of monosaccharide components are recorded using differential refractmeter. The proportion of each monosaccharide can be determined from standard curve prepared using standard sugars. Concentration of sugars, in the gum sample, is estimated from a peak height-concentration curve constructed using standard sugars (Anderson *et al*., 1983).

3.2.11. Determination of cationic composition

Dry ash method was used in sample preparation where2 grams of gum sample was placed in a well-glazed porcelain dish, and then heated to 550^oC, maintained at this temperature for 4 hours. The sample was cooled and 10 ml. of 3N HCl was added, covered with watch glass, and sample boiled gently for 10 minute, cooled, filters in to 100 ml. volumetric flasks, and diluted to the volume with deionized water. Atomic absorption spectrometer (model Sens AA, GBC

scientific equipment PtyLtD) was used to determine seven elements namely K, Na, Ca, Mg, Fe, Zn and Cu.

3.3. Preparation of glucuronic acid

glucuronic acid was prepared using column chromatography method where a glass column backed with an amberlite resin IR 120 H+ (a strong cation exchange resin) was used. Gum solution (20%) was prepared (on dry weight basis). After the sample was completely dissolved, the solution was left until it became free from bubbles and insoluble matter before proceeding to further steps. The solution was passed slowly through the column in order to replace its cations by the hydrogen bonded to the resin; the collected eluent was glucuronic acid. The pH of the glucuronic acid solution was measured using a pre calibrated pH meter (ORP electrode, USA). The column was regenerated again by washing with 10% solution of hydrochloric acid followed by deionized water until it became free from chloride ion (Adams *et al.*, 1977).

3.4. Preparation of glucuronates

Glucuronates were prepared by ion exchange method using column chromatography. A solution of salts was prepared by dissolving 1.2 g. of each of potassium, calcium, magnesium and sodium chloride which equal to the specific amount of these cations present in *Acacia senegal* under study. The salts solution was passed through the column until it was saturated by cations, followed by deionized water until it became free from chloride ion. 20% glucuronic acid solution was passed slowly through the column in its salt form, the collected eluent was glucuronates. The solution of glucuronic acid and glucuronates were dried using Freeze Dryer (Modulo; Edwards England); (Schleif *et al*., 1951) presented in Figure (3.1). The samples were dried through evaporation under high pressure. The

moisture removed from samples under vacuum without passing through a liquid stage (sublimation). The temperature used from -100 to +100^{o}C and the Vacuum range was from -10 to 5 atm. The final product moisture content was in the range of 1-2%.

3.5. Morphological examination and determination percentage of *Acacia senegal*, glucuronic acid and glucuronates

3.5.1. Theory

A scanning electron microscopy (SEM) is a type of electron microscope that produces images of a sample surface by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum, in low vacuum, and in environmental SEM in wet conditions (Almuslet *et al*., 2012)

3.5.2. Preparation of the sample

Fine powder of the samples were prepared by making a thin film of desirable size and mounted on copper stubs with double – side's adhesive tape and coated with gold using Sputter Coater S150A Edwards – England. The specimens were examined under JXA – 840A Electron Probe Microanalyzer – JEOL – JAPAN shown in Figure (3.2).

3.6. Equivalent conductivity measurements

Stock solutions (20%) glucuronic acid and glucuronates (freeze dried) were prepared .Six solutions were prepared from stock solution (0.1, 0.05, 0.02, 0.01, 0.005, 0.002); equivalent conductivity measurements were made with a conductivity cell of the PL-700 series bench top multi-parameters type. The cell constant was 0.02 cm^{-1}.The water used had a specific conductivity of 1.2µS cm^{-1} (Nelson *et al*., 1971, Rios *et al*., 1993). The instrument used in this experiment presented in Figure (3.3).

3.7. Rheological measurement

Rheological measurements were carried out using (Bohlin Rheometer CS, UK) shown in Figure (3.4) with software and instrument (KTB 30) fitted with cone and plate geometry with a cone diameter of 40 mm and face angle of 4^0. Steady shear viscosity curves were measured for *Acacia senegal* gum, glucuronic acid and glucuronates solutions upon shear rate ramp-up (from 1 to 1600 s^{-1}) and subsequent shear rate ramp-down (from 1600 back to 1 s^{-1}). The temperature used is isothermal at 25^{o}C, the applied time is 10s. at linear range. Dynamic rheological measurements were performed to determine the elastic modulus (G′) and viscous modulus (G″) at 25^0C. The experiment carried out in frequency range of 0.1–10 Hz. The rheometer control and data processing was done by dedicated computer software.

Figure (3.1): Typical Freeze Dryer Modulyo Edwards (England)

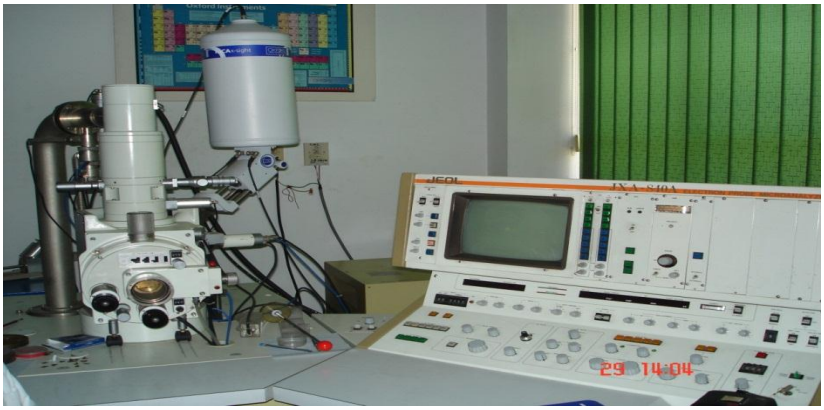


Figure (3.2): Typical JXA-840A Electron Probe Microanalyzer-JEOL-JAPAN.

Figure (3.3): Conductivity cell of the PL-700 series new-GOnDO bench top multi-parameters type

Figure (3.4): Bohlin rheometer

3.7.1. Solution preparation

25% w/w (based on dry weight) solutions of *Acacia senegal* gum, glucuronic acid and glucuronates were prepared in water containing 0.005 % w/v NaN_3 as a preservative. The solutions agitated on a tube roller mixer overnight to ensure that the sample fully dissolves and hydrated. The solutions were then centrifuged for 15 minutes at speed of 1500 rpm using (Beckman J_2 – HS, USA) centrifuge. The solutions were then stored at 4^oC till investigate of their rheological behavior.

3.8. Determination of emulsification properties

3.8.1. Emulsion preparation

Emulsion of *Acacia senengal* gum, glucuronic acid and glucuronates were prepared with the following concentrations: orange oil 6.5%; *Acacia senegal*,

glucuronic acid and glucuronates 20%; sodium benzoate as preservative 0.1%; citric acid 0.4% and deionized water 73.4% (Buffo, 2001).

Solutions of 25% (w/w) were prepared from *Acacia senengal* gum, glucuronic acid and glucuronates by hydrating them over night and gently mixing until completely dissolved. Pre-emulsion were prepared by adding all the components (sodium benzoate, citric acid and orange oil); the additional water to lower gum concentration to 20% w/w and to dissolve the sodium benzoate and homogenizing with a bench-top high-speed mixer (Wise Tis model Tis Tis, 26.000 rpm, USA) for 5 minutes. Fine emulsifications were achieved by subjecting the pre-emulsions to a high pressure homogenizer Ultrasonicator (JEIOTECH, UC – 10, UK).

3.8.2. Droplet size analysis

The droplet size distributions of the emulsions prepared were analyzed as freshly prepared using Zeta Sizer Nano Zs (Malvern Instruments, UK), the instrument is presented below in Figure (3.5). Distilled water was used as dispersant. The prepared emulsions samples were kept closed in a glass vessel at 60^{o}C and the particle size distribution of the emulsions were determined using Zeta Sizer Nano Zs at time intervals 3 and 7 days.

Figure (3. 5): Zetasizer Nano ZS (Malvern Instruments).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1. Physicochemical properties of *Acacia senegal* var. *senegal* gum.

Acacia gums have variety of uses; regarding to the commercial interest and importance of the gums as food additive, physicochemical characterization of them, is of great importance.

A number of physicochemical and chemical methods were used to characterize *Acacia senegal* var. *senegal* gum, these methods included determination of moisture, ash, specific optical rotation, intrinsic viscosity, pH, nitrogen and protein content, acid equivalent weight, uronic acid anhydride, number average molecular weight, refractive index, sugar and cationic composition. The analytical data of the sample are presented in Table (4.1) below.

Table (4.1): Physicochemical properties of *Acacia senegal* var. *senegal* gum.

Moisture%	8.42	**Acid equivalent weight**	1239.6
Ash%	3.5	**M_n($\times 10^5$Da)**	4.1
Optical rotation $[\alpha]_D^T$ (-)	24.87	**Refractive index**	1.336
Viscosity (ml/g^{-1})	12.002	**Arabinose (%)**	33.79
pH	4.6	**Galactose (%)**	40.70
Nitrogen%	0.35	**Rhamnose (%)**	09.31
Protein%	2.23	**Glucouronic acid (%)**	15.65

The moisture content of *Acacia senegal* var. *senegal* gum sample used in this study, was 8.42 as shown in Table (4.1). The result show that the moisture content of the sample fall within the usual range of study done by Karamalla *et al.*, (1998) to moisture content of 803 *Acacia senegal* gum samples collected in season 1994/1995 who reported the moisture range of 8.1% – 14.05%.The result is close to the values reported for *Acacia senegal* gum by Younes (2009) which was in the range of 9.91% – 14.72% and to the values reported by Karamalla in his study to *Acacia senegal* and *Acacia seyal* gums collected between1960 – 1999 in Sudan which was in the range of 10.75 -9.4 % respectively. The result is different from those mentioned by (Randal *et al.*, 1988; Osman *et al*., 1993 and Idris *et al.*., 1989); which were 15.5%, 12 - 15 %, 12.5 - 16 % respectively.

The value of ash content was found to be 3.5 as shown in Table (4.1). The result is similar to those reported by Yebeyen (2009) in his study of *Acacia senegal* as to be 3.56%. Also the result was in a good agreement with the values of ash content determined for 731 *Acacia senegal* gum samples collected in season 1994/1995 by Karamalla *et al*., (1998) which were found in the range of 2.75 – 5.25%. The maximum limit of total ash for food and pharmaceutical quality gum arabic is set to 4% w/w (Chikamai, 1996; FAO, 1999; Karamalla, *et al*., 1998; Mhinzi, *et al*., 1995), and this is in a good agreement with the result obtained in the present study, which was 3.5%.

The specific optical rotation is considered as the most important criterion of purity and identity of any type of gum. *Acacia senegal* gum has negative specific optical rotation and belongs to *Vulgares* series that contains *Acacia Leata, Acacia polyacantha, Acacia mellifera*….etc. The result of specific optical rotation which shown in Table (4.1) is -24.87°; agree with the results obtained by Jurasek *et al.*, (1993), he reported the specific optical rotation of *Acacia senegal* gum to be in the

range -20° to -32°.The value of optical rotation is less than the values given by (FAO, 1990; Osman, 1993; Idris *et al*., 1998; Karamalla, 1999; and Abdelrahman, 2008).

The viscosity of plant gums is an important commercial property, it is a factor involving the size and the shape of the macromolecules and the conformation they adopt in the solvent .The value of the viscosity of the gum under study is shown in Table (4.1) above and Figure (4.1) below. The result is in good agreement with that reported by Al-Assaf *et al*., (2005) and is within the range of the study undertaken by Idris *et al*., (1998). It could be conclude, that, *Acacia* exudates are not, particularly, viscous when compared with gums from other botanical genera such as Prunus, Combreturm, stericulia (karaya), Astragatus (tragacanth). Recent studies (Fincher, *et al*., 1983) indicated that: the low viscosity of gum arabic is due to its globular and closely –packed molecular structure.

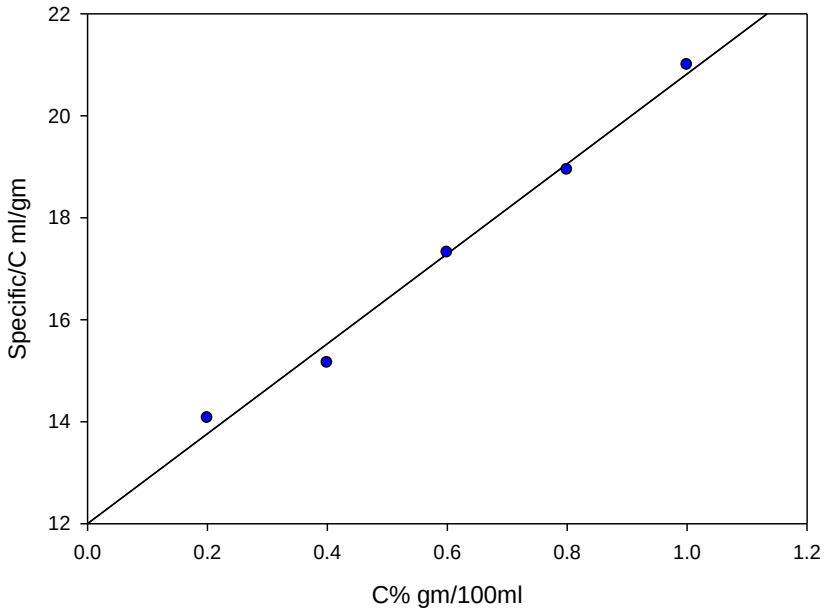


Figure (4.1): Intrinsic viscosity of *Acacia senegal* var. *senegal* gum

The evaluation of the molecular weight characteristics in routine quality control is very important since gum arabic is an industrially important product. In emulsion and suspension technology, its ability to stabilize and increase viscosity is, directly, dependent on molecular weight, the lower the molecular weight the less effective it is at a given concentration resulting in a less stable preparation. In tableting its binding characteristics are also dependent on molecular weight, the binding power increasing with molecular weight. In addition the molecular weight of gum arabic have an adverse effect on tablet disintegration, drug dissolution and bioavailability as the molecular weight of acacia increases its rate of hydration and solubility decreases and its viscosity, binding and protective power increases Alain *et al*., (1985).

The value of number average molecular weight (M_n) is shown in Table (4.1) above and calculated osmatic pressure is illustrated in Figure (4.2). The result obtained compare with the result obtained by Idris *et al*., (1998) is the same and also closed to the results given by Saverborn (1944) and Swenson *et al*., (1968).

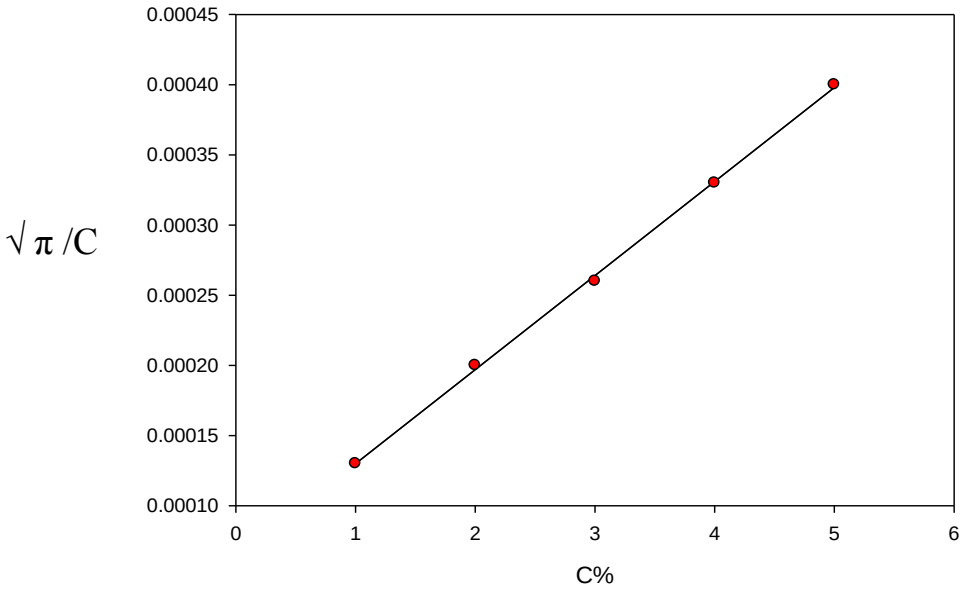


Figure (4.2): Osmatic pressure of *Acacia senegal* var *senegal* gum

The pH value of *Acacia senegal* var. *senegal* gum is shown in Tables (4.1). The value is found to be 4.6, was in good agreement with the results citied in the literature (Karamalla *et al*., (1998).

The percentage of nitrogen and protein content of crude *Acacia senegal* gum are shown in Table (4.1) which were calculated using kjeldahl method. They were 0.35 and 2.31 for nitrogen and protein respectively. The total protein content was calculated using nitrogen conversion factor (NCF) of 6.6 resulting from amino acid analysis. The results is in the range reported by Idris *et al*.,(1998) which were 0.22 - 3.90% in his study of *Acacia senegal* trees having different ages. The value is close to the results obtained by Karamalla (1998) and Elmanan *et al*., (2008) which were in the range of 0.328% to 0.27 - 0.33% nitrogen content for *Acacia senegal* gum respectively. The result of nitrogen content is typical to Yebeyen *et al*., (2009) in his study on *Acacia senegal* gum in central rift valley of Ethiopia; he reported the value of 0.35. The result of nitrogen content is higher than that obtained for *Acacia seyal* gum by Anderson *et al*., (1963); reported the value of 0.09 - 0.19.

. The acid equivalent weight and corresponding calculated glucouronic acid content are given in Table (4.1). The result shows that the acid equivalent weight and glucouronic acid of *Acacia senegal* gum samples are 1239.6 and 15.65 respectively. The result is ingood agreement with the value mentioned in the literature (Karamalla 1999; Idris *et al*., 1998; Osman *et al*., 1993 and Siddig *et al*., 2005).

The refractive index increment of *Acacia senegal* var. *senegal* gum is shown in Table (4.1) and was found to be 1.336cm^3/g. The result given is very

different from those reported in the literature 0.141 cm^3/g by Randall *et al*., (1989) and also is very higher than the value reported by Anderson *et al.*, (1969), which was 0.146 cm^3/ g. The reason of this disagreement may be due to the instruments used in the studies or to the kind of the solvent used or even to the condition of the experiment done (Swenson *et al*., (1968).

The sugar contents of *Acacia senegal* var. *senegal* gum were given in Table (4.1). The results were found to be 33.79%, 40.70%, 9.31 and 15.65% for arabinose, galactose, rhaminose and glucouronic acid respectively. The results fall in the range of values reported in literature for *Acacia senegal* gum by Jurasek *et al.,* (1993) who reported 23 – 35%, 34 – 46% and 09- 16% for arabinose, galactose and rhaminose respectively .The value were also close to the results obtained by Karamalla (1999) which were 24 – 29%, 36 – 42% and 12 – 14 for arabinose, galactose and rhaminose respectively. The value of galactose resembles the result mentioned by Anderson *et al*., (1966) for galactose composition which was 41%.

From the above mention results we can notice that galactose has the highest percentage value in *Acacia senegal* gum where in *Acacia seyal* arabinose is high than the other sugars (Islam *et al*., (1997); Osman *et al*., 1993; Siddig *et al*., 2005; Abdelrahman; 2008).

The values of cationic composition of *Acacia senegal* var. *senegal* gum sample were presented in Table (4.2) below. *Acacia senegal* gum showed cationic content trend as follows Ca > K > Mg > Na > Fe > Zn > Cu. The results obtained in this study show that calcium, potassium, and magnesium recorded the high values among cations studied, this indicates that the gum is a salt of calcium, potassium, and magnesium. Characterization of gum arabic by mineral content is not common in most of the literatures cited. However, the Ca, K, Mg and Na content of the present *Acacia senegal* gum sample are 172.3, 129.9, 47.33 and

6.156 in ppm. respectively, is close to the values reported by Anderson, (1990) and also not very different from the results reported by the same researcher in his results reported in 1989. The results of minerals content also close to values reported by Younes, (2009). The amounts of heavy metals such as Pb have to be minimal, and it is set as less than 2 ppm (FAO, 1999); hence *Acacia senegal* gum analysis in this study show better results, since the value of heavy metals were at trace levels when compared with the international standards set for gum arabic as food additives reflecting the high quality of the gum derived from this part of the gum belt.

Table (4.2): Cationic composition of *Acacia senegal* var. *senegal* gum in ppm.

Elements	Values in ppm.
Ca	172.3
K	129.9
Mg	47.33
Na	6.156
Fe	0.693
Zn	0.261
Cu	0.183

4.2. Modification of *Acacia senegal* var. *senegal* gum.

Natural gums are preferred over comparable synthetic materials due to their non-toxicity, low cost and availability. Most of the natural gums are safe enough for oral consumption in the form of food additives or drug carriers. However, there are certain problems associated with the use of gums. These include uncontrolled rates of hydration, pH dependent solubility, thickening, drop in viscosity on

storage, and the possibility of microbial contamination. Chemical modification of gums not only minimizes these drawbacks but also enables their use for specific drug delivery purposes (Rana *et al*., 2**0**11)**.**

4.2.1. Calculations of cationic composition of *Acacia senegal* gum

The cationic values in Table (4.2) above were used to calculate the a mounts of the Ca, K, Mg and Na cations present in *Acacia senegal* gum used in this study. The amounts given are 172300 µg/g of ash, 128900 µg/g of ash, 47330 µg/g of ash and 6156 µg /g of ash, for calcium, potassium, magnesium and sodium respectively.

According to Anderson (1990); the amount of cations can be calculated as follows:

Total of basic cations = 172300+128900+47330+6156**=354686 µg/g of ash.**

The ash content of *Acacia senegal* under study is 3.5% hence, if 100 g gum produces 3.5 g of ash that means one gram produces 0.035 g of ash.

Therefore total of basic cations (µg/g gum) = 354686 × 0.035 **= 12414.01 µg/g gum.**

Glucuronates solution was prepared by dissolving 12414.01 **µg /g gum** which equal to the total amount of Ca, K, Mg and Na present in *Acacia senegal*. The ions were integrated successfully to the chain of the glucuronic acid. The solution of glucuronic acid and the glucuronates were dried to the powder form with the final product of moisture 1- 2% using freezed dryer.

4.2.2. Scanning electron microscopy (SEM) of *Acacia senegal* gum, glucuronic acid and glucuronates

Figures (4.3) to (4.8) show morphological examination of *Acacia sensgal* gum, glucuronic acid and glucuronates which carried out using (SEM).The specimens were examined under JXA – 840A Electron Probe Microanalyzer – JEOL – JAPAN.

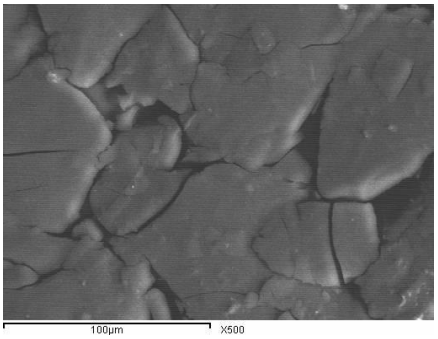


Figure (4.3): Typical SEM images of *Acacia senegal*

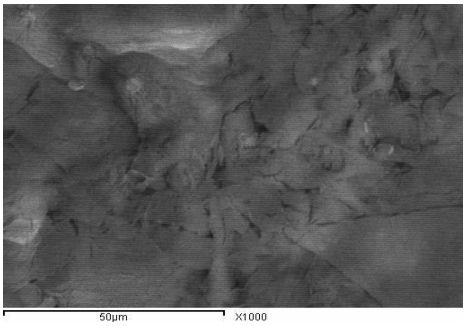


Figure (4.4): Typical SEM images of glucuronic acid

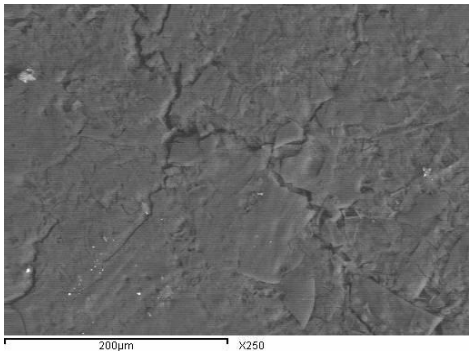


Figure (4.5): Typical SEM images of sodium glucuronate

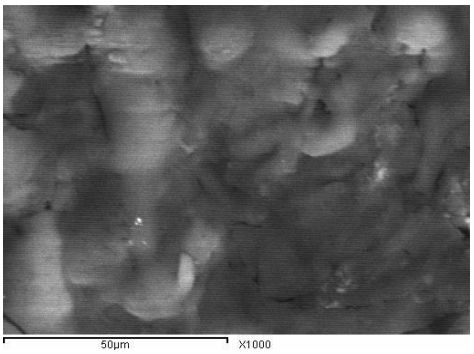


Figure (4.6): Typical SEM images of potassium glucuronate

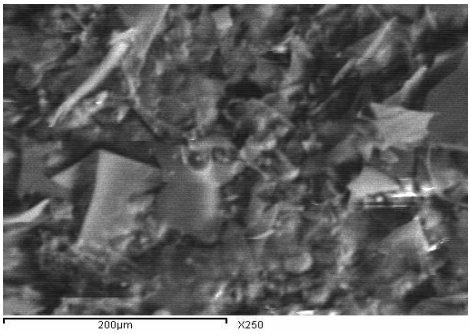


Figure (4.7): Typical SEM images of calcium glucuronate

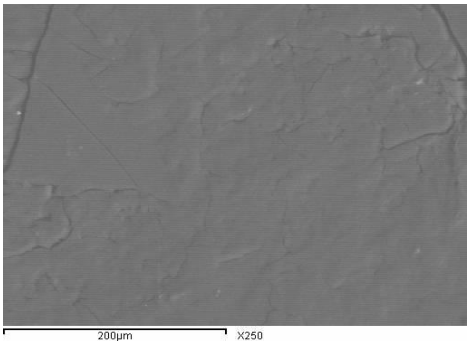
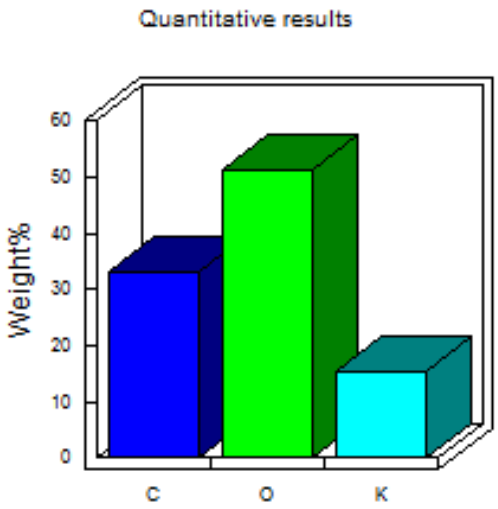


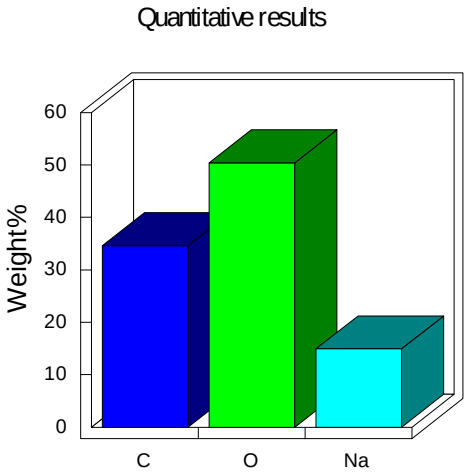
Figure (4.8): Typical SEM images of magnesium glucuronate

It was observed that the surface of glucuroniac acid and glucuronates are clearly different from each other and they were all together different from the parent *Acacia senegal* gum, which has a rocky surface. The surface evidence supports successful integration of the cations to the glucuronic acid molecule. SEM also determines the weight percentage of the cations present in the samples.

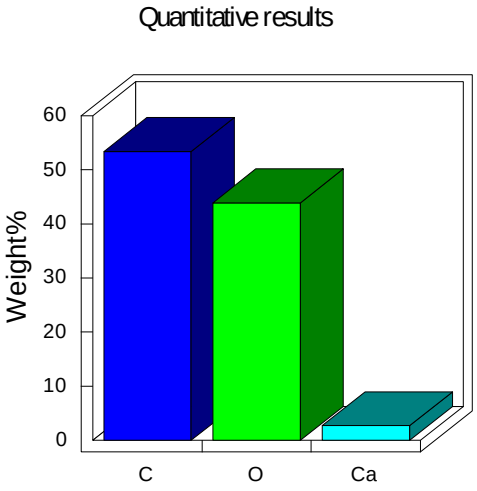
Figure (4.9) show quantitative data of weight percentage of Na, K, Ca and Mg in glucuronic acid which give additional evidence, to supports successful integration of the cations into the glucuronic acid molecule.



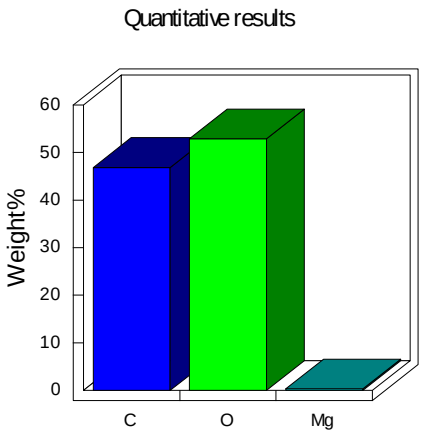
(a) Potassium glucuronate



(b) Sodium glucuronate



(c) Calcium glucuronate



(d) Magnesium glucuronate

Figure (4.9): weight percentages of cations in glucuronic acid

4.3. Properties of glucuronic acid and glucuronates

4.3.1. Viscosity determination

Gum arabic, from which the glucuronic acid under investigation was derived, is an example of a colloid with a negative charge. This charge is due to the carboxyl groups in the molecule. It would be expected, therefore, that a charge would exist on molecules of glucuronic acid and its salts as it does on gum arabic.

Previous reports (Schleif, *et al* 1951; Wood, 1954) dealt with preparation of glucuronic acid in the powdered form by the spray-drying process. In this work, glucuronic acid and its salts were prepared in powder form using freezed – drying technique.

Glucuronic acid, Na, K, Ca and Mg glucuronates show insignificant variation in the pH from parent gum as shown in Table (4.3), calcium and magnesium glucuronates showed close value of pH. Na and K glucuronates were in a similar pH range; glucuronic acid show typical acid behaves with low pH. Table (4.3) and Figure (4.10) show intrinsic viscosity of *Acacia senegal* gum, glucuronic acid and glucuronates in1M NaCl. The results show that glucuronates have smaller viscosity than that of glucuronic acid and the parent gum. It was observed that the viscosity of glucuronic acid solution is decreased with the addition of metal ions and it was further decrease with increasing concentration of NaCl. Self-suppression of the charge by the colloidal particles of the solution may give explanation for the decrease in viscosity. The results are in good agreement with those reported by Schleif (1951).

Table (4.3) intrinsic viscosity of *A. senegal*, glucuronic acid and glucuronates

Sample	PH	[η] in 1M NaCl	[η] in 0.5 M NaCl	[η] in deionized water
***A. senegal* gum**	4.6	12.002	15.21	22.34
Glucuronic acid	2.6	08.53	10.82	18.42
Na glucuronate	6.9	02.47	15.18	45.82
K glucuronate	6.5	0.12	12.97	30.72
Ca glucuronate	4.9	0.05	10.10	11.63
Mg glucuronate	4.5	-1.23	09.29	10.83

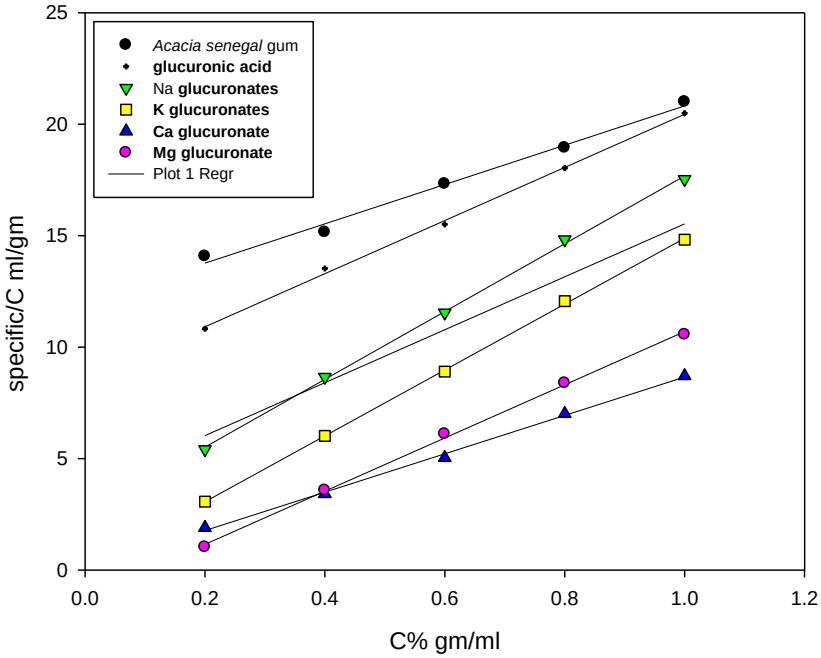


Figure (4.10): Viscosity of *Acacia senegal*, glucuronic acid, Na, K, Ca and Mg glucuronates in 1M NaCl solution

Figure (4.11) and Table (4.3) show intrinsic viscosity of *Acacia senegal* gum, glucuronic acid and glucuronates in 0.5 M NaCl. It was observed that as concentration of NaCl solution decrease the viscosity of the glucuroniates increase 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 interaction that make the network more compact and formed (Oliveira 2001).

Figure (4.12) and Table (4.3) show intrinsic viscosity of *Acacia senegal* gum, glucuronic acid and glucuronates in deionized water. The results show an increase in the viscosity of Na and K glucuronates as well as Ca and Mg glucuronates. The rapid increase in the viscosity of glucuronates has been attributed to the charge on the molecule (electro-viscous effect) which affects the viscosity to a large extent (Schleif 1951). It was also observed that electrolyte added to the colloidal solutions will suppress the charge, resulting in a curve which is linear.

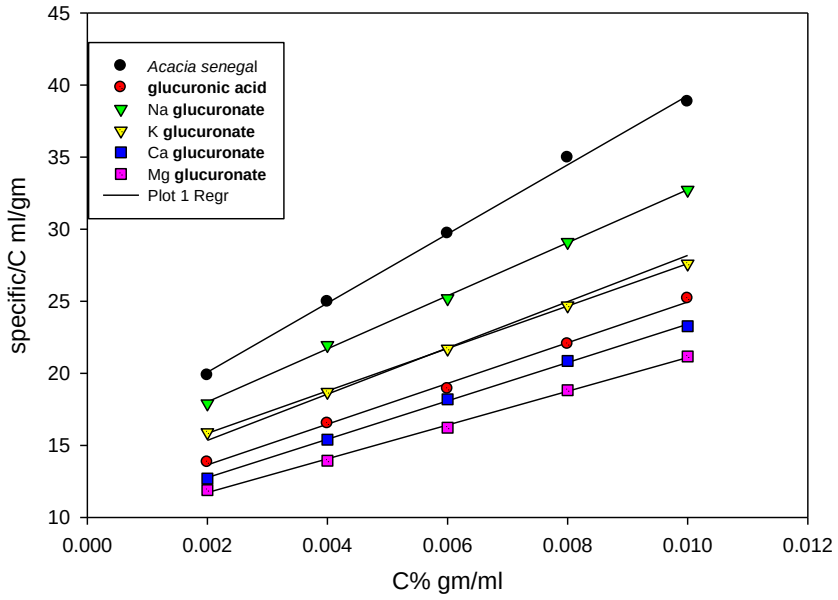


Figure (4.11): Viscosity of *Acacia senega*l gum, glucuronic acid, Na, K, Ca and Mg glucuronates in 0.5M NaCl solution

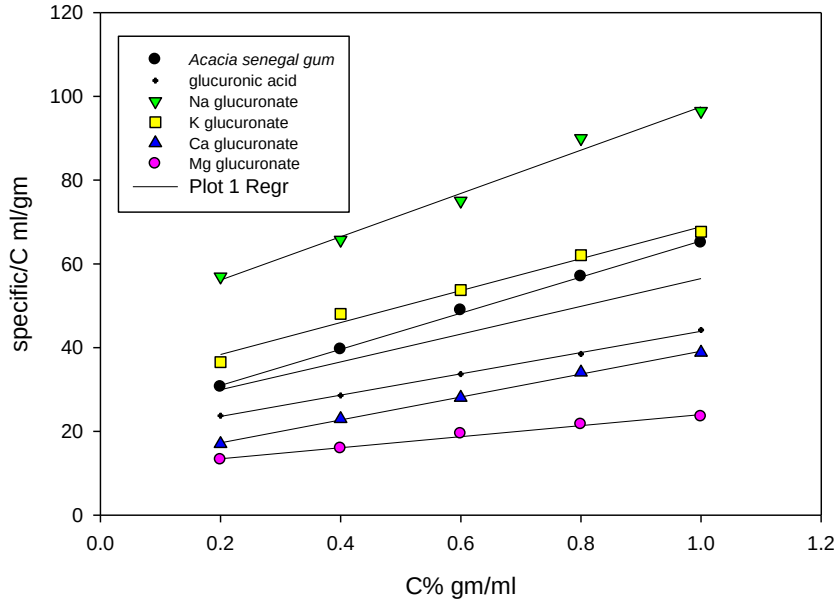
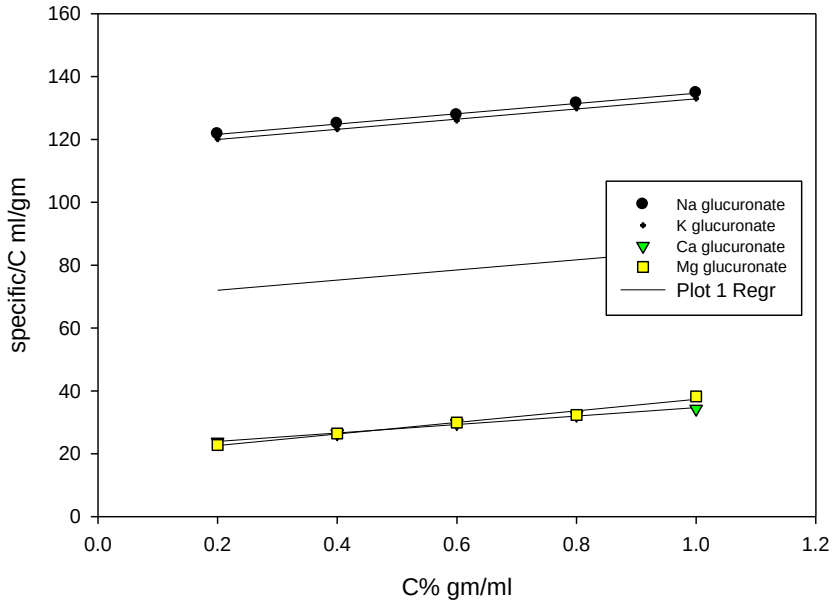


Figure (4.12): Viscosity of *Acacia senegal*, glucuronic acid, Na, K, Ca and Mg glucuronates in deionized water

Table (4.4) and Figure (4.13) show intrinsic viscosities of Na, K, Ca and Mg glucuronates at pH 5.5 (food preparation setting) and low concentration (3%) plotted as reduce viscosity vs. concentration. By comparison, Na and K glucuronates have the highest viscosity, while the glucuronic acid has the lowest (Sloniewsky, 1971). It was observed that a considerable difference exists in the viscosity curves of sodium glucuronate and glucuronic acid without the presence of NaCl salt and pH adjusted to 5.5. One possible explanation is that the higher viscosity of sodium glucuronate was due to a higher charge on the molecule. Colloids with carboxylic acid groups lose their negative charge quite readily at low pH, but in the region of neutrality ionization is practically complete, thus the charge would be high**.** The potassium glucuronate curve is the same in magnitude and appearance as the sodium glucuronate curve. In the case of the divalent salts the curve were much lower than the monovalent glucuronates and also do not exhibited rapid increase in viscosity, as shown by the monovalent glucuronates at ow concentrations. Apparently, instead of an intermolecular attraction linking the carboxyl groups of two molecules, an intramolecular attraction occurred effectively linking the carboxyl groups on one molecule, thus making the molecule more compact, resulting in less hydration and lowered viscosity. The curve for the magnesium glucuronates is the same in magnitude and appearance as the curve for the calcium glucuronate.

Table (4.4): intrinsic viscosity of glucuronates in pH 5.5 and 3% concentration.

Sample	[η]
Na glucuronate	118.37
K glucuronate	116.73
Ca glucuronate	21.27
Mg glucuronate	19.03



Figure(4.13): Viscosity of Na, K, Ca and Mg glucuronates in PH 5.5 and 3% concentration.

4.3.2. Equivalent conductivity of glucuronic acid and glucuronates.

Equivalent conductivity for glucuronic acid, Na, K, Ca and Mg glucuronates at 25^{o}C are determined using a conductivity cell of the PL-700 series bench top multi-parameters type. Figure (4.14) show the results of equivalent conductivity as a function of the square root of the equivalent concentration for glucuronic acid, Na, K, Ca and Mg glucuronates. The results are the same as other polyelectrolytes results which were determined by Nelson (1971).The equivalent conductivities show a sharp increase with dilution and thus cannot be extrapolated to infinite dilution. However, the curve for each glucuronates shows a distinct minimum before the sharp increase. K glucuronate show a maximum at very high dilution while magnesium salt gives minimum conductivity among glucuronates.

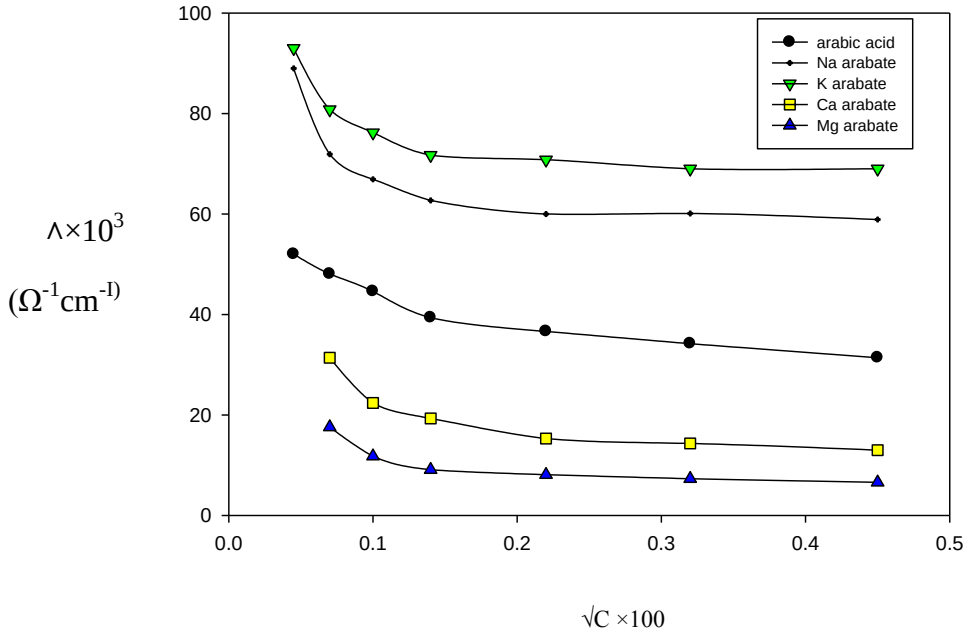


Figure (4.14): Equivalent conductivity of glucuronic acid, Na, K, Ca and Mg glucuronates in deionized wate

4.4 Rheological properties of *Acacia senegal* gum, glucuronic acid and glucuronates.

4.4.1. Shear flow viscosity

Figure (4.15) show variation of shear stress with shear rate for *Acacia senegal* var. *senegal* gum, glucuronic acid and glucuronates at concentration of 25% w/v and Figure (4.16) show viscosity-shear rate profile. In comparison; *Acacia senegal* gum plot show Newtonian behaviour while glucuronic acid show non-Newtonian behavior (Pseudoplastic) and hence it is shear thinning. All glucuronates resemble gum arabic in their behaviour; they are Newtonian with linear relationship between stresses and shear rate where the proportionality constant is viscosity. From Figure (4.16) Ca and K flow behavior are typical as the flow behavior of gum *Acacia senegal.* The result of *Acacia senegal* gum given is the same as the results reported by some authors such as Rao, *et al*.; 1999; Tanaka, *et al*.; 2006. It has been reported that aqueous solution of gum arabic has generally been considered to behave as a Newtonian fluid due to the highly branched AG component. Nevertheless, a few studies have observed shear thinning at low shear rates (Li *et al*., 2011).

Gum arabic solutions show Newtonian flow behavior in the shear rate range from 50 s^{-1} to 100 s^{-1}, even at gum arabic concentration as low as 4%. In the shear rate range from 1 s^{-1} to 50 s^{-1}, the shear thinning behavior has been observed. In concentration range between 10 and 25%, gum arabic solutions show a significant shear thinning behavior, as compared with that of solutions with concentration in the range 30-50 % (Tanaka, 2006).

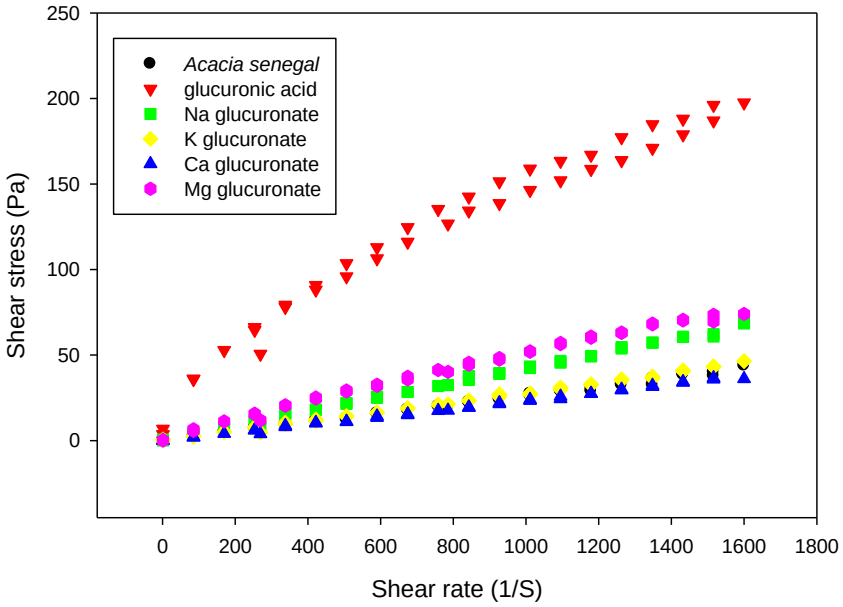


Figure (4.15): Shear stress - shear rate profile for *Acacia senegal* gum, glucuronic acid, Na, K, Ca and Mg glucuronates at 25% concentration

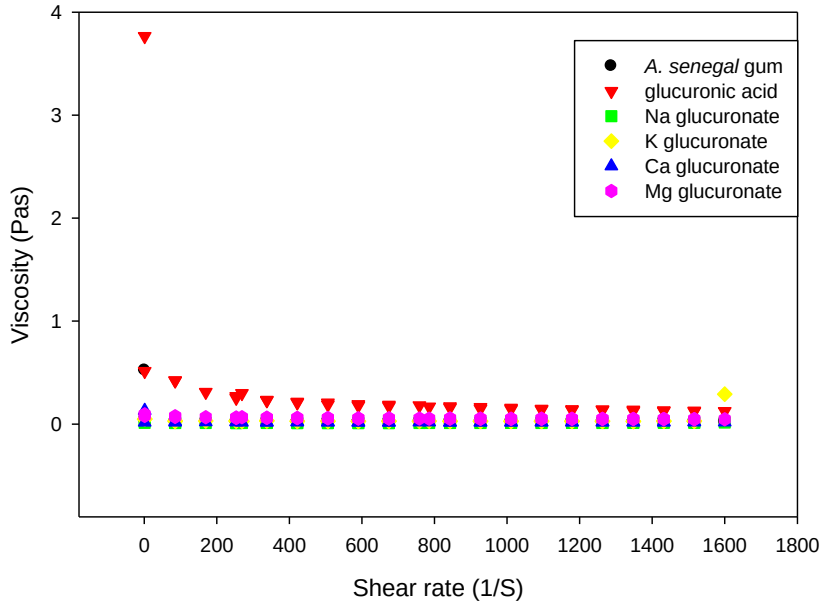


Figure (4.16): viscosity - shear rate profile of *A. senegal*, glucuronic acid, Na, K, Ca and Mg glucuronate at 25% concentration

4.4.2. Dynamic rheological behavior

Figures (4.17) to (4. 21) show variation of G' and G" with frequency of oscillation for concentration (25% w/v) at 25° C for *Acacia senegal* gum, glucuronic acid, Na, K, Ca and Mg glucuronates respectively. All figures show a higher G″ than G′ in the lower region of the frequency range tested, i.e. G″ is dominating G′ in the lower region of the tested oscillatory deformation regime, and hence the viscous behaviour of the *Acacia senegal* gum and glucuronates dominates the elastic behaviour. After certain frequency a cross-over of G' and G" is seen; these cross-over values of *Acacia senegal* and glucuronates indicate to their viscoelastic properties in this region (Ramachandran, *et al.*, 1999). As frequency increase G′ is become greater than G″ throughout the tested oscillatory deformation regime for *Acacia senegal* gum and glucuroniates, i.e. the elastic behaviour of the *Acacia senegal* gum and glucuronates dominates the viscous behaviour and this indicate that there is changes in the behaviour of *Acacia senegal* gum and glucuronates from viscous to viscoelastic to elastic properties which suggest different structural type being presented (Seo, *et al*., 2012).

Figures (4.22) show variation of G' and G" with frequency of oscillation for concentration (25% w/v) at 25°C for glucuronic acid. In comparison glucuronic acid is quite different in behavior than *Acacia senegal* gum and glucuronates, as figure show G' and G" profiles are parallel (no cross-over). G′ is greater than G″ throughout the tested oscillatory deformation regime, i.e. the elastic behaviour of the glucuronic acid dominates the viscous behaviour.

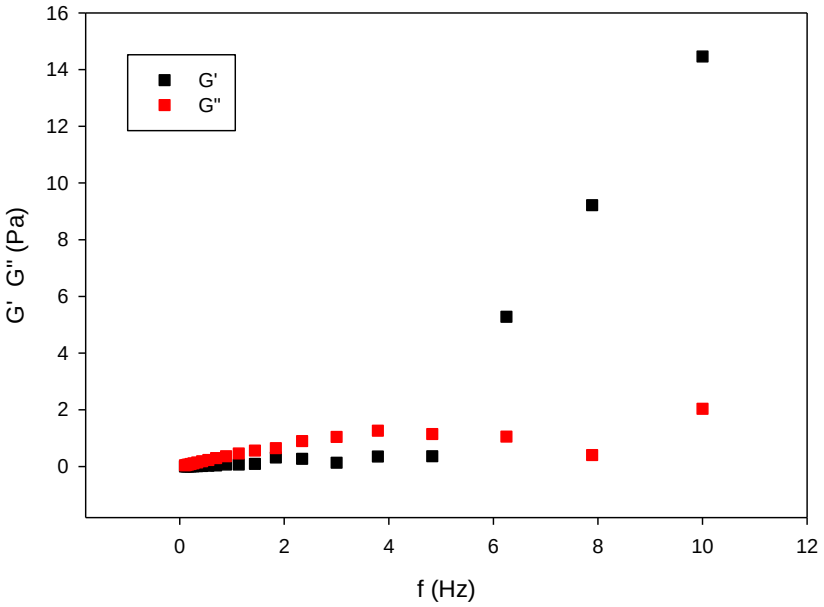


Figure (4.17):Storage modules G' and loss modulus G'' under oscillatory deformation regime for 25% (w/v) *A. senegal* var. *senegal* gum at 25 C.

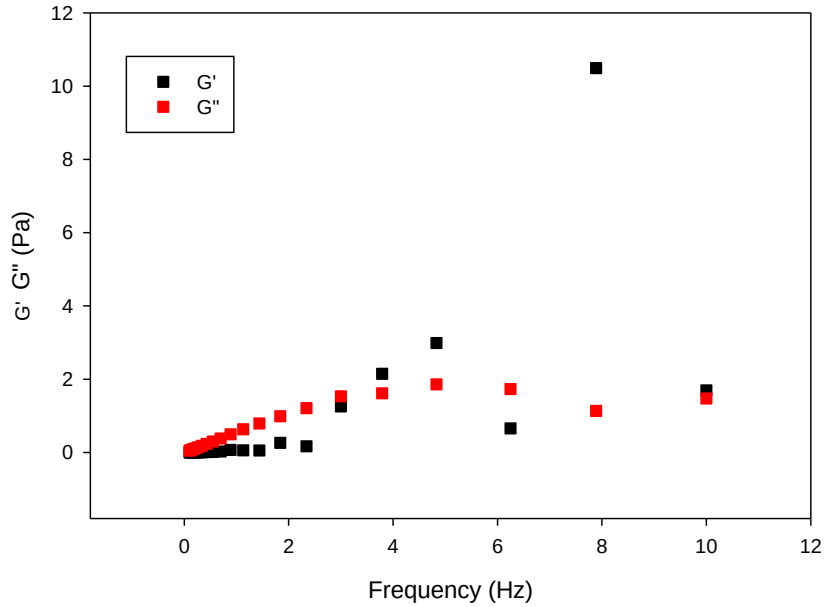


Figure (4.18):Storage modules G' and loss modulus G'' under oscillatory deformation regime for 25% (w/v) for Na glucuronate

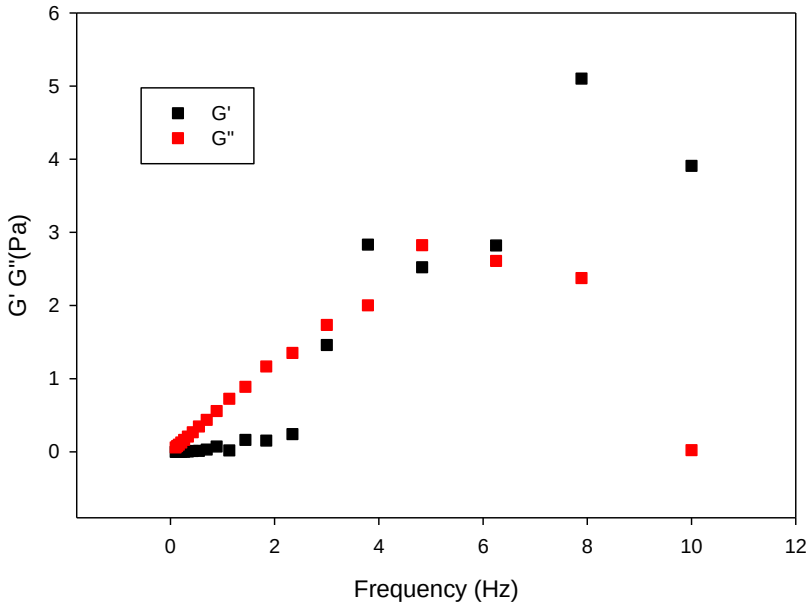


Figure (4.19):Storage modules G' and loss modulus G" under oscillatory deformation regime for 25% (w/v) for k glucuronate

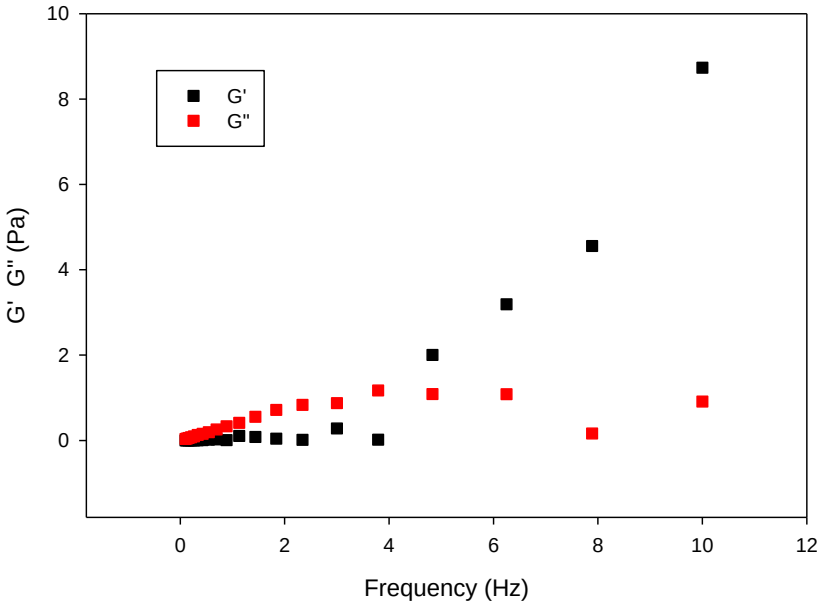


Figure (4.20):Storage modules G' and loss modulus G" under oscillatory deformation regime for 25% (w/v) for Ca glucuronate

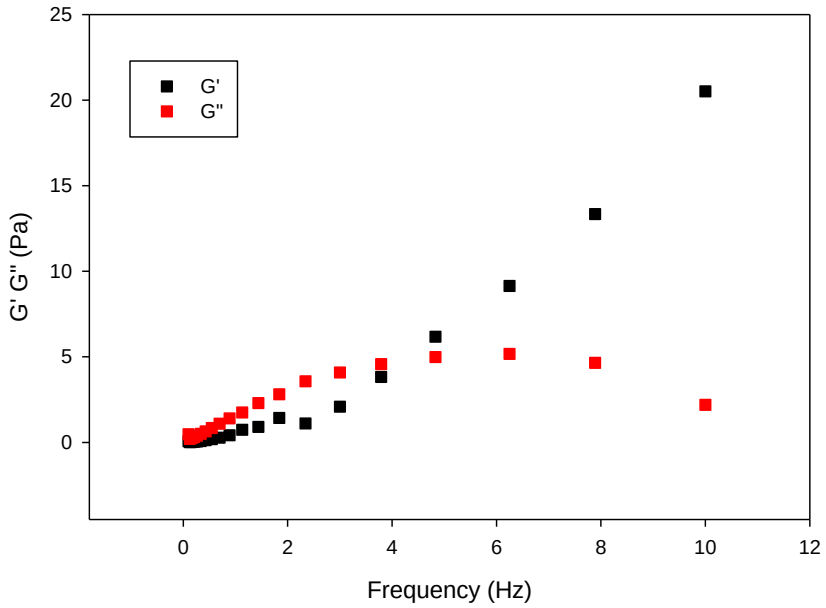


Figure (4.21):Storage modules G' and loss modulus G'' under oscillatory deformation regime for 25% (w/v) for Mg glucuronate

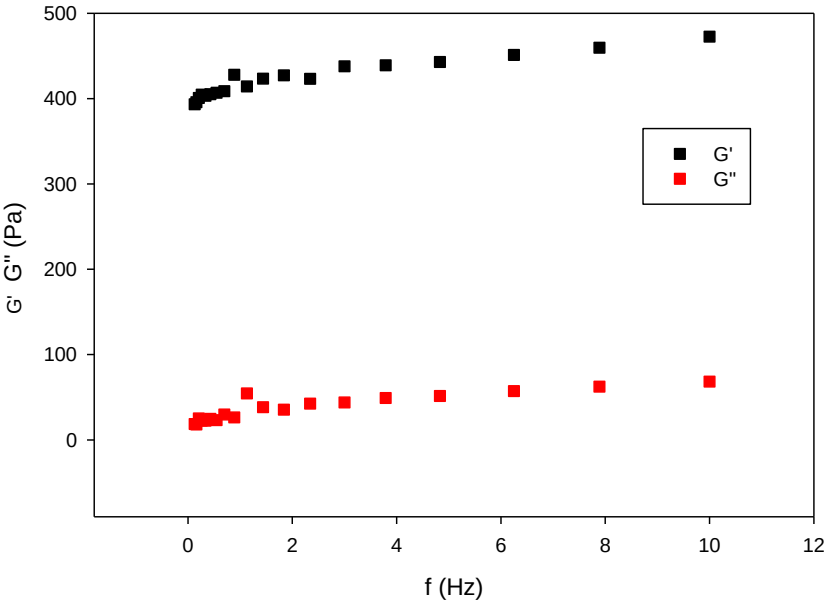


Figure (4.22):Storage modules G' and loss modulus G'' under oscillatory deformation regime for 25% (w/v) for glucuronic acid

4.5. Emulsification properties of *Acacia senegal* gum, glucuronic acid and glucuronates.

The particle size distribution for freshly prepared emulsion and after 3 and 7 days was measured using Zeta Sizer Nano Zs (Malvern Instruments, UK), a particle size distribution analyzer.

Table (4.5) and Figures (4.23) to (4.28) show the average size of the emulsion droplet for *Acacia senengal* gum, glucuronic acid and glucuronates at different conditions (fresh emulsion and after storage for 3 and 7 days at 60^{o}C respectively). Results showed that change was found in most emulsions during the incubation for 7 days at 60^{o}C. The emulsion of Na, Ca and Mg salts show stability until 3 days while *Acacia senengal* gum forms multiple peaks from 3 days and completely separated in 7 days. The emulsion of K and glucuronic acid results clearly showed that no change was found in most emulsions during the incubation for 7 days at 60^{o}C which means that they are most stable over the all.

The emulsifying properties study of the gum arabic glucuronic acid and glucuroniates show clearly that K glucuronate and glucuronic acid are form more stable emulsion than *Acacia senegal* gum and Na, Ca and Mg glucuroniates. The order of stability is glucuronic acid, K, Na, Ca, Mg, and *Acacia senegal* gum.

The explanation for the stability of glucuronic acid refer to very small size of glucuronic acid which make the molecule very compact and coating; in addition to the fact that the present of hydroxyl groups in glucuronic acid molecule are responsible for the production of oil-in- water emulsion.

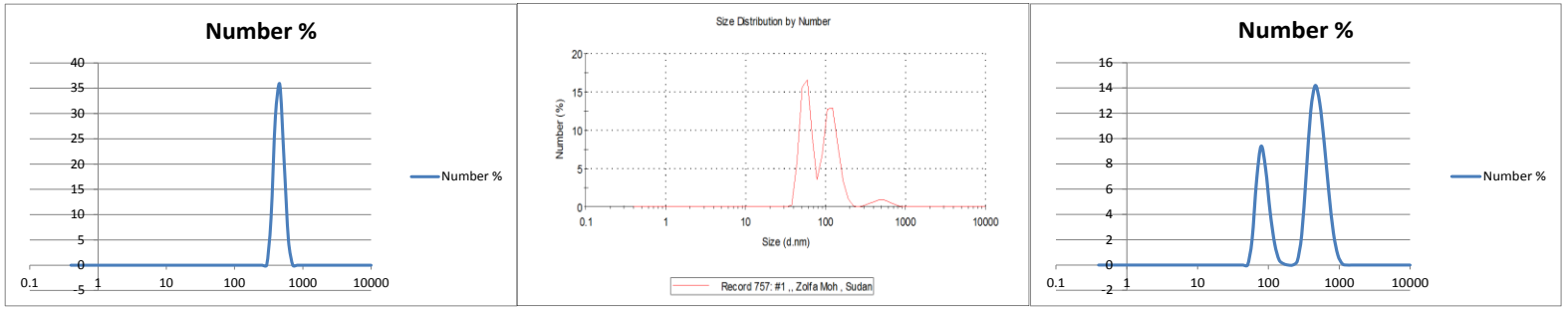
When the cross-section **o**f the hydrocarbon chain of an emulsifying agent, and the metallic end of the molecule are of the same magnitude, there is little or no

tendency to curvature and hence, no stable emulsion will result. With the univalent glucuroniates mainly in the case of K glucuronates 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. Also the optimum size of K glucuronate compared with other cations is additional reason. For Na glucuronate completely face separation was notice after 7 days. However, when a divalent metal attacked to glucuronic acid molecule, the hydroxyl groups increase the magnitude of the hydrocarbon chain that there is little tendency to curvature, and the emulsions obtained are less stable and this obtained by Ca and Mg glucuroniates in this study. For *Acacia senegal* gum it is begin to form 3 peaks from 3 days and this refer to its arabino galacto protein (AGP) component which responsible for its unequalled emulsifying properties.

The emulsifying powers for all samples as show in Table (4.5) are equal but the emulsifying stability is different among them with time.

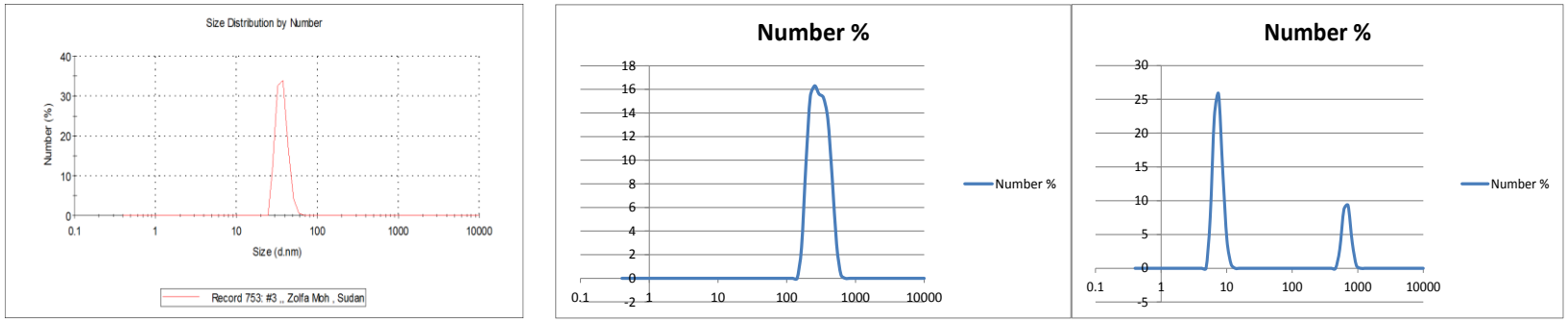
Table (4.5): Average size of the emulsion droplet in (nm) of *A. senegal* gum, glucuronic acid and glucuronates at different conditions.

Samples	Freshly prepared	After 3 days at 60°C	After 7 days at 60°C
***Acacia senegal* gum**	700	Peak 1 700 Peak 2 150 Peak 3 60	Peak 1 700 Peak 2 80
Na glucuronate	700	500	Peak 1 750 Peak 2 7
K glucuronate	700	700	700
Ca glucuronate	700	700	Peak 1 750 Peak 2 100
Mg glucuronate	740	700	Peak 1 700 Peak 2 250
Glucuronic acid	700	700	600



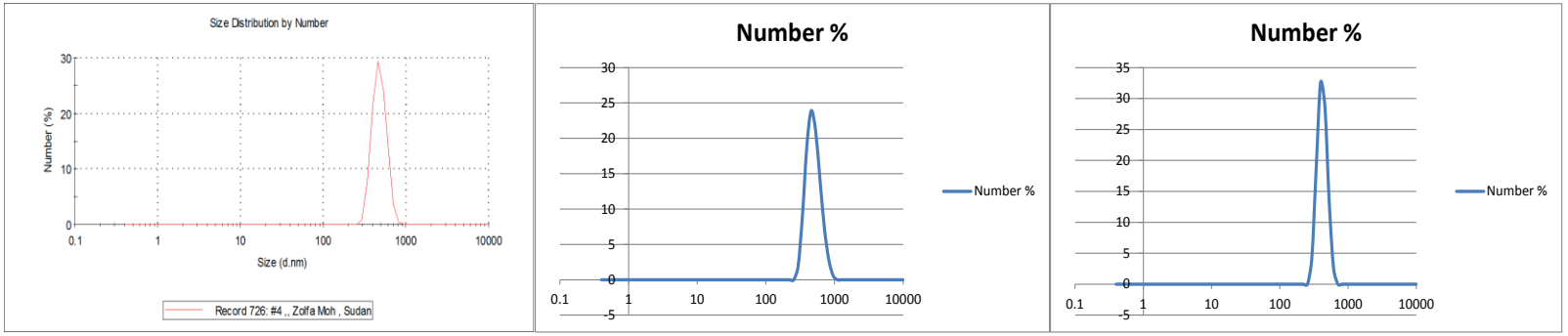
(a) Freshly prepared (b) after 3 days (C) after 7 days

Figure (4.23): Particle size distributions of *Acacia senegal* gum emulsion as freshly prepared, stored for 3 and 7 days at 60^0C.



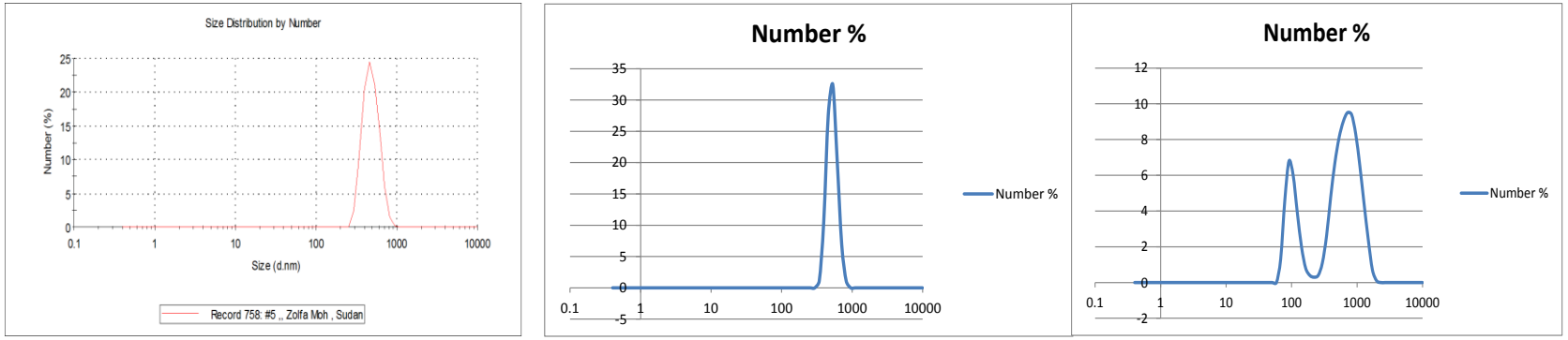
(a) Freshly prepared (b) after 3 days (C) after 7 days

Figure (4.24): Particle size distributions of Na glucuronate emulsion as freshly prepared, stored for 3 and 7 days at 60^0C.



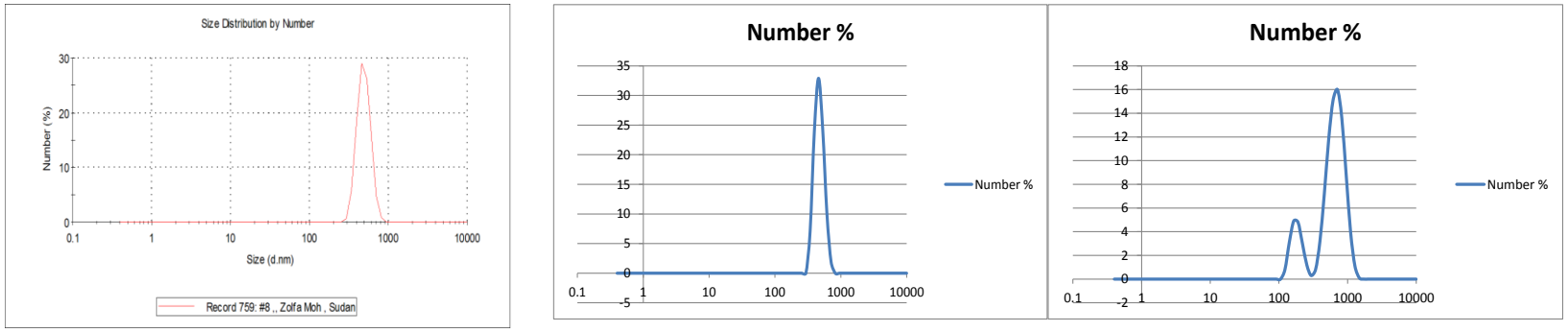
(a) Freshly prepared (b) after 3 days (C) after 7 days

Figure (4.25): Particle size distributions of K glucuronate emulsion as freshly prepared, stored for 3 and 7 days at 60^0C.



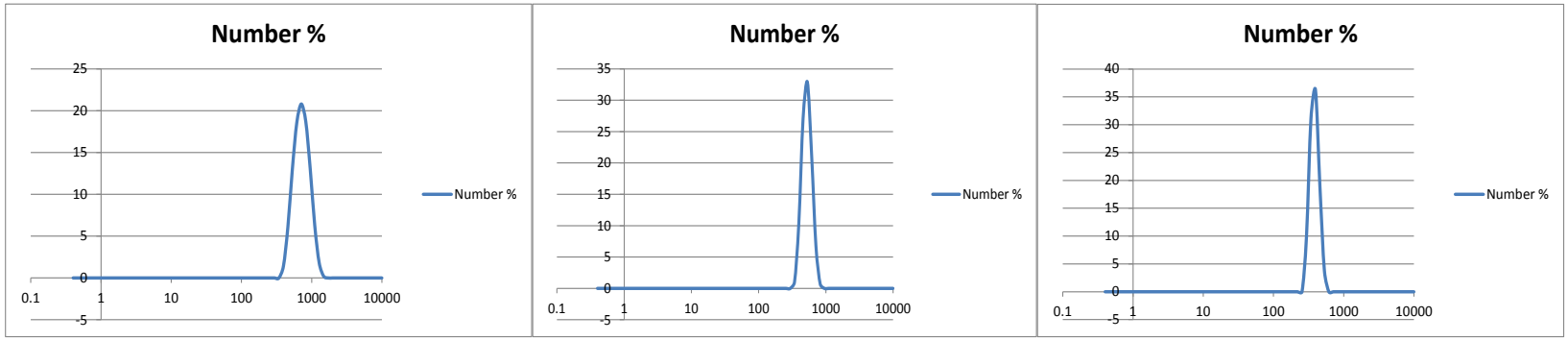
(a) Freshly prepared (b) after 3 days (C) after 7 days

Figure (4.26): Particle size distributions of Ca glucuronate emulsion as freshly prepared, stored for 3 and 7 days at 60^0C.



(a) Freshly prepared (b) after 3 days (C) after 7 days

Figure (4.27): Particle size distributions of Mg glucuronate emulsion as freshly prepared, stored for 3 and 7 days at 60^0C.



(a) Freshly prepared (b) after 3 days (C) after 7 days

Figure (4.28): Particle size distributions of glucuronic acid emulsion as freshly prepared, stored for 3 and 7 days at 60^0C.

Summary and conclusions:

The physicochemical study of *Acacia senegal* var. *senegal* gum show the followings characterizations:

The average value of moisture, ash, specific rotation, intrinsic viscosity, pH, nitrogen, protein, number average molecular weight, acid equivalent weight and refractive index of *Acacia senegal* gum were found to be 8.42%, 3.5%, -24.87°, 12.002 ml g^{-1}, 4.6, 0.35%, 2.23%, 4.1×10^5 Da ,1239.6 and 1.336 respectively.

The average values of galactose, arabinose, rhamanoe and glucuronic acid for *Acacia senegal* gum, the values were found to be 40.70, 33.79, 9.31 and 15.65 respectively.

The cationic composition study shows that calcium has the highest value followed by, potassium, magnesium and sodium

pH values were 2.6, 6.9, 6.5, 4.9, 4.5 for glucuronic acid, Na, K, Ca and Mg glucuronate respectively.

The measured viscosities were 8.53, 2.47, 0.11, 0.05 and -1.24 ml g^{-1} for glucuronic acid, Na, K, Ca and Mg glucuronates respectively in 1M NaCl and 15.21, 10.82, 14.33, 12.9, 10.12 an d 9.38 ml g^{-1} for *Acacia senegal* gum, glucuronic acid, Na, K, Ca and Mg glucuronates respectively in 0.5M NaCl where they were 22.34, 18.42, 45.82, 30.72, 11.76 and 10.83 ml g^{-1} for *Acacia senegal* gum, glucuronic acid, Na, K, Ca and Mg glucuronates respectively in deionized water. The intrinsic viscosity in dilute 3% concentration and pH of 5.5 were 118.37, 116.73, 21.27 and 18.91 ml g^{-1} for Na, K, Ca and Mg glucuronates respectively.

The equivalent conductivities showed very sharp nonlinear increase with decreasing polyelectrolyte concentration.

The rheological study show Newtonian behavior for *Acacia senegal* gum and glucuronates while glucuronic acid exhibit Non - Newtonian behavior. *Acacia senegal* gum and glucuronates are viscous with $G' < G''$ while glucuronic acid is elastic with $G' > G''$

The emulsifying properties study of the gum arabic, glucuronic acid glucuronates showed clearly that K glucuronates and glucuronic acid are form more stable emulsion than *Acacia senegal* gum, Na, Ca and Mg glucuronates. The order of stability is glucuronic acid, K, Na, Ca, Mg glucuronates and finally *Acacia senegal* gum

Recommendations:

Suggestions of further work for the *Acacia senegal* gum and glucuronates. Includes:

- Determination of the structural feature and properties of glucuronates when two or three cations introduced.
- Study the toxicological properties of glucuronates.
- Study the thermodynamic parameters of glucuronates.

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