Sudan University of Science and Technology

College of Graduate Studies

Air fractionation and physicochemical properties of the fractions from *Acacia polyacantha* gum

التجزئة الهوائية والخصائص الفيزوكيميائية لصمغ الكاكموت واجزاءه

A dissertation Submitted in Partial Fulfillment of the

Requirements for M.Sc. Degree in Chemistry

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٢٠١٦

Holy Quran Verse

قالالله تعالي :

((أَلَمْ تَرَكَيْفَ ضَرَبَ اللَّهُ مَثَلًا كَلِمَةً طَيِّبَةً كَشَجَرَةٍ طَيِّبَةٍ أَصْلُهَا ثابِتٌ وَفَرْعُهَا فِي السَّمَاءِ * تُؤْتِي أُكُلَهَا كُلَّ حِينٍ بِإِذْنِ رَبِّهَا وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ لعَلَّهُمْ يَتَذَكَّرُونَ * وَمَثَلُ كَلِمَةٍ خَبِيثَةٍ كَشَجَرَةٍ خَبِيثَةٍ اجْتُثَّتْ مِنْ فَوْقِ الْأَرْضِ مَالَهَا مِنْ قَرَارٍ *يُثَبِّتُ اللَّهُ الَّذِينَ آَمَنُوا بِالْقَوْلِ الثَّابِتِ فِي الْحَيَاةِ الدُّنْيَا وَفِي الْآَخِرَةِ وَيُضِلّ ُ اللَّهُ الظَّالِمِينَ وَيَفْعَلُ اللَّهُ مَا يَشَاءُ))

[إبراهيم: ٢٤- ٢٧].

Dedication

I dedicate this work to ……..

My father……

My mother……

My family with love ……

Acknowledgement

Praise to Allah who gave me health and patience to accomplish this work. I would like to express my gratitude to Dr. Mohammed Elmobark Osman for supervision, advices and guidance from the very early stage of this research as well as giving me, extraordinary, experience throughout the work, Above all and the most needed, he provided me unflinching encouragement and support in various ways. his truly scientist intuition has made him as a constant oasis of ideas and passions for science, which , exceptionally ,inspired and enriched my growth as a student ,researcher and scientist .I am indebted to him more than he knows.

I would like to thank my sisters and brothers for their financial support; my deepest gratitude goes to my parents who, sincerely, raised me with their caring and gentle love.

Finally, I would like to extend my thanks to my colleagues and friends in Sudan University of Science and Technology and to, all, those who have helped and inspired me during my work.

Abstract

In this study, Acacia polycantha gum solution (12.7%) was fractionated by a foaming method using aerating pump into two fractions .The first is the hydrophobic fraction(HO) which is high protein fraction and the second is the solution remaining after the fractionation operation which has been freeze drying (hydrophilic fraction) .then an analytical study on fractions was done to explain physical and chemical properties such as nitrogen content , protein content, ash content, moisture content, intrinsic viscosity, PH value, number average molecular weight , specific optical rotation, and refractive index. Also spectroscopic study was done to determine cationic composition such as sodium, calcium, magnesium, potassium and iron. At last comparative the results of the fractions with previous study for crude *polyacantha* gum and the results as following:

A proximate analysis of two fractions(HO , HI) and crude gum respectively revealed that, moisture content(10.29, 8.4 and ,7.5%),whereas the ash content (2.85 ,3.4and 3%) , specific rotation(24-, -18 and- 17.3),refractive index(1.685, 1.685, 1.337) and nitrogen content (0.56, 0.35,and0.35%)were significantly different. Protein content(3.6, 2.31 and, 2.28%), PH value(4.65, 3.1 and 4.9) and the intrinsic viscosity (0.7807, 0.422 and 10.3ml/g)were, highly, significantly different. Also number average molecular weight for tow fractions obtain from osmometer results (HI fraction =148,274 g/mole) and (HO fraction =76,310g/mole) ,while the average molecular weight for crude gum = 136,900 g/mole ,the high molecular weight and high solubility were indicate that *Acacia polyacantha* gum is high branched . Also UV absorption spectra of two fractions were determined and maximum were found to be a proximately the same, that indicates similar chemical structure. Cationic composition of two fractions was studied. Na, K and Ca by flame photometer, Fe and Mg by atomic absorption spectroscopy , the results obtained(K) is the highest value(HI =130.125 , HO = 109.05µg/g)Followed by Ca (HI = 100.92 , HO=90.99µg/g) , Na (HI= 39.75, HO= 7.107µg/g), Mg (HI =14.915 , HO =15.392µg/g) and Fe (HI = 2.4031, HO = 1.583µg/g) , while the crude gum contains high of Na and Fe than value in fractions and low value in K ,Mg and Ca .

المستخلص

في هذه الدراسة تم تجزئية محلول صمغ الكاكموت(12.7%)بطريقة فصل الرغوة عن طريق مضخة الهواء(aerating pump) الي متجزئين الأول هي الرغوة (Hydrophobic fraction) وهي الاعلي في محتوي البروتين والثاني هو المحلول المتبقي بعد عملية الفصل اجريت له عملية تجفيد (Hydrophilic fraction) ثم اجريت دراسة تحليلية علي المتجزئات لتوضيح الخواص الفيزيائية والكيميائية وهي نسبة النيتروجين ، البروتين ، الرماد ، الرطوبة ،اللزوجة الضمنية ،الرقم الهيدروجيني،متوسط الوزن الجزيئي العددي ،الدروان النوعي ومعامل الانكسار.كذلك اجريت دراسة طيفية لتحديد تراكيزبعض العناصر المعدنية مثل الصوديوم،البوتاسيوم ،الكالسيوم ،الماغنزيوم والحديد ،اخيرا تم اجراء المقارنة بين النتائج المتحصل عليها مع نتائج دراسة سابقة لصمغ الكاكموت الخام وكانت النتائج للمتجزئين (HI),(HO) وصمغ الكاكموت الخام علي التوالي كالتالي :

نسبة الرطوبة (7.5,8.4,10.29) %، نسبة الرماد (3،3.41,2.85) % ، الدوران النوعي (24-,12-,17.3-)ومعامل الانكسار (1.337,1.685,1.685) ، محتوي النيتروجين (0.56 ,0.35,0.35)%،محتوي البروتين(2.28,2.31,3.6)%، الرقم الهيدروجيني (4.65,.2.9, 4.9) واللزوجة الضمنية (0.78 ,0.422 ,10.3).g/ml

تم حساب متوسط الوزن الجزئي العددي للمتجزئين من نتائج الاوزموميتر وكانت(HI fraction = 148,274 g/mole)،(HO fraction =76,310 g/mole) وبينما كان الوزن الجزئي العددي للصمغ الخام هو 136.900g/mol يستنتج من الوزن الجزئي العالي والذوبانية العالية في الماء ان جزئي الكاكموت علي درجة عالية من التفرع . كذلك عرضت العينات للاشعة فوق البنفسجية وكانت اقصي نقاط الامتصاص للمتجزئين متماثلة تقريبا مما يدل علي تشابه التركيب الكيميائي .كذلك تمت دراسة محتوي المعادن للعينات تحت الدراسة باستخدام جهاز مطياف اللهب الضوئي لتقدير كل من الكالسيوم ،الصوديوم والبوتاسيوم وجهازمطياف الامتصاص الذري لتقديرالحديد والماغنزيوم .اوضحت الدراسة ان البوتاسيوم يظهر اعلي قيمة(HI=130 , HO =109µg/g) يليه الكالسيوم (HI=100, HO=90µg/g,)، صوديوم (HI=39 , HO=7.1µg/g) ، ماغنزيوم (HI=14, HO=15µg/g)،الحديد (HI =2.4 , HO=1.5µg/g) بينما نجد ان الصمغ الخام اعلي قيمة في محتوي الصوديوم والحديد مما هو موجودفي المتجزئين تحت الدراسة Fe, µg/g (Na =43 =28µg/g) واقل في محتوي البوتاسيوم، الكالسيوم والماغنزيوم.

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Chapter one

Literature Review

1.1Introduction

Sudanese major gums of economic importance are in the order of Gum Arabic, Gum Talha and *polyacantha* gum. Gum Arabic, sometimes known as the dried gummy exudation of *Acacia*(JECFA,1990). Gum refers to any polysaccharide that is dispersible in water to make a viscous solution, gels or colloidal dispersions(Anderson1994). Generally gums are long chain, high molecular weight, polymers that dissolve or disperse in water to give thickening gelling effect and exhibit related secondary functional properties, such as emulsification, stabilization, and encapsulation(Sharma1981). Gums, or hydrocolloids, are mainly long – chain, straight to branch polysaccharides that contain hydroxyl groups that can bond to water molecules. These chains consist of (2000 to 10000) monosaccharide's units; the sugars monomers may contain linked side units, or substituent groups, such as sulphates, methyl ethers, esters and acetates. Gums composed mainly of C, H, O and N elements,and acidic gums(e.g.Gum Arabic) contain mainly Ca, Mg, Na and Fe as Cations(Elnour,2007). Specifications are quality indices which are set to characterize the nature of the gums, since gum Arabic is the most important species used in food products and a wide range of other industrial applications. According to Anderson(1986), in order to identify a particular gum from a series of different gum exudates an extensive number of analytical tests have to be performed. This approach enable a finger print of each gum to be determined, The currently analytical test used include determination of the total ash, nitrogen (hence protein), measurements of optical rotation ,intrinsic viscosity , equivalent weight, analysis neutral sugars(arabinose, rhamnose and galactose) and glucuronic acid content after hydrolysis and measurement of molecular mass. Since international trade requires vigorous quality control specifications to identify the product and trace any adulteration processes, it is essential that firm parameters are established for the gum property of gum Arabic. According to American Food Chemical Codex, the specification for identity and purity of gum Arabic were published in 1978(Karamalla, 1998), and it has been reviewed every 4 years(JECFA, 1982, 1986, 1990,and 1995) in 1990(JECFA, 1990) significant changes e.g. range for specific rotation(-26.0º to -34.0º) and nitrogen content(0.27 to 0.39%) were introduced. However, in 1995 JECFA further recommended that specific rotation and nitrogen content be deleted. The mostly important property of a gum which makes it unique amongst polysaccharides generally it is solubility and viscosity. The majority of gums dissolve in water at different concentration(e.g. Gum Arabic can form solutions of up to 60% forming viscous solutions) .These properties of gums can be utilized in many applications , such

as in the food industry which is the major one, where emulsifying and stabilizing properties are utilized. The gum is also used in the pharmaceutical and medical fields, in addition to other industries (cosmetic,adhesive, paints and inks). Gum from *Acacia polyacantha* tree is a dried exudation obtained from stem and branches of *Acacia polyacantha* tree, Kakamut tree, is widely, distributed in Africa throughout tropical Africa. In Sudan, there are several regional varieties, which usually occur along rivers and valleys where the water table is fairly high and soils are clay. In Sudan *Acacia polyacantha* tree is widely spread in Upper Nile Province, Kordofan Province and Blue Nile Province. The wood is used mainly in fuel and charcoal of good quality, fence posts, farm implements and railway sleeper, beams, and rafters. The gum is edible and used as adhesive in the treatment of textile fibers. The roots are used to act as a general, health tonic as antidote for snake bite, and cure for venereal diseases. A preparation from the bark is used for general stomach disorders(Omeret al.,2015)

1.2 Name derivation

The term *Acacia* comes from the Greek *akantha,* meaning thorn. The whole tree is covered with dark brown to black hooked thorns that are paired. On the stem they are shorter and more rigid than on the branches. For this reason, the tree was named *polycantha*, which means many thorns. The subspecies name, *campylacantha* means curved thorns(Baker, 1956)

1.3 Definitions

Kakamut gum is the dry exudate obtained from the stems and branches of *Acacia polyacantha* var. *campylacantha*(L) Willdenow(*fam.Leguminosae*). It consists, mainly, of salts of an acidic arabino-galactan protein complex, which on hydrolysis yields galactose, arabinose, rhamnose, glucuronic acid and 4-O-methyl glucuronic acid. Botanical Classification of *Acacia polyacantha* gumis:

Family	*Leguminosae*
Subfamily	*Mimosaceae*
Genus	*Acacia*
Species	*Polyacantha*
English name	*Flacons claw Acacia*
Arabic name	Kakamut,
Order	*Fabales*
Class	*Magnoliopsida – Dicotyledons* (El Amin, 1977 &Voget, 1995).

1.4 Distribution

All African *A. polyacantha* is placed in the subspecies *campylacantha*. The tree often grows up in in the moist, subtropical bush-veld of Africa, usually in alluvial soils near rivers. It is widespread in tropical Africa, Figure(1.1), occurring from Gambia to Ethiopia and southwards to Kenya and Zimbabwe; it has a limited distribution in South Africa, being found only in Limpopo; some specimens can be found in the Kruger National Park, in the Punda Maria and Pafuri areas. The typical subspecies is referred to only from India and Srilanka in Asia. In Sudan, there are several regional varieties which usually occur along rivers and valleys where the water table is fairly high, and the soils are suitable (Heba. A.N, 2009)

1.5Description

The tree, occasionally, reaches 20 m in height and the trunk can be 70cmin diameter. Knobby bark and paired thorns are its most conspicuous features. The bark is yellowish with bone scale and black knots in the place of former leaves and thorns. Thorns occur in pairs and are sharply, curved. They are brown with black tips of leaves may reach 25cm in length, are imparipinnate with 10- 40 pairs of pinnae and 35 -60 of leaflets each. Prominent gland occurs at the base of the leave. Flowers grow in pairs or 3 spicate racemes from the leaf axial and are cream colored and strongly scented. Fruits consist of pods up to 15cm long, which each contains 5-9 seeds, Figure (1.2)

1.6Origins of plant gums

The origin of the plant gum is still uncertain, but it is thought by some authorities to be the starch granules present in the cells. Great many plants exude viscous, gummy liquids, which when exposed to air and allowed to dry, clear, glassy masses, gums are worthy of investigation in their own right and as an aspect of plant biochemistry in bacterial polysaccharides. They occur naturally as salts (especially of calcium and magnesium) and in some cases a proportion of the hydroxyl groups are etherified, most frequently as acetates. The site of esterification is, however, entirely unknown at present structurally gums are related to other less complex plant polysaccharides(Omer, 2004).

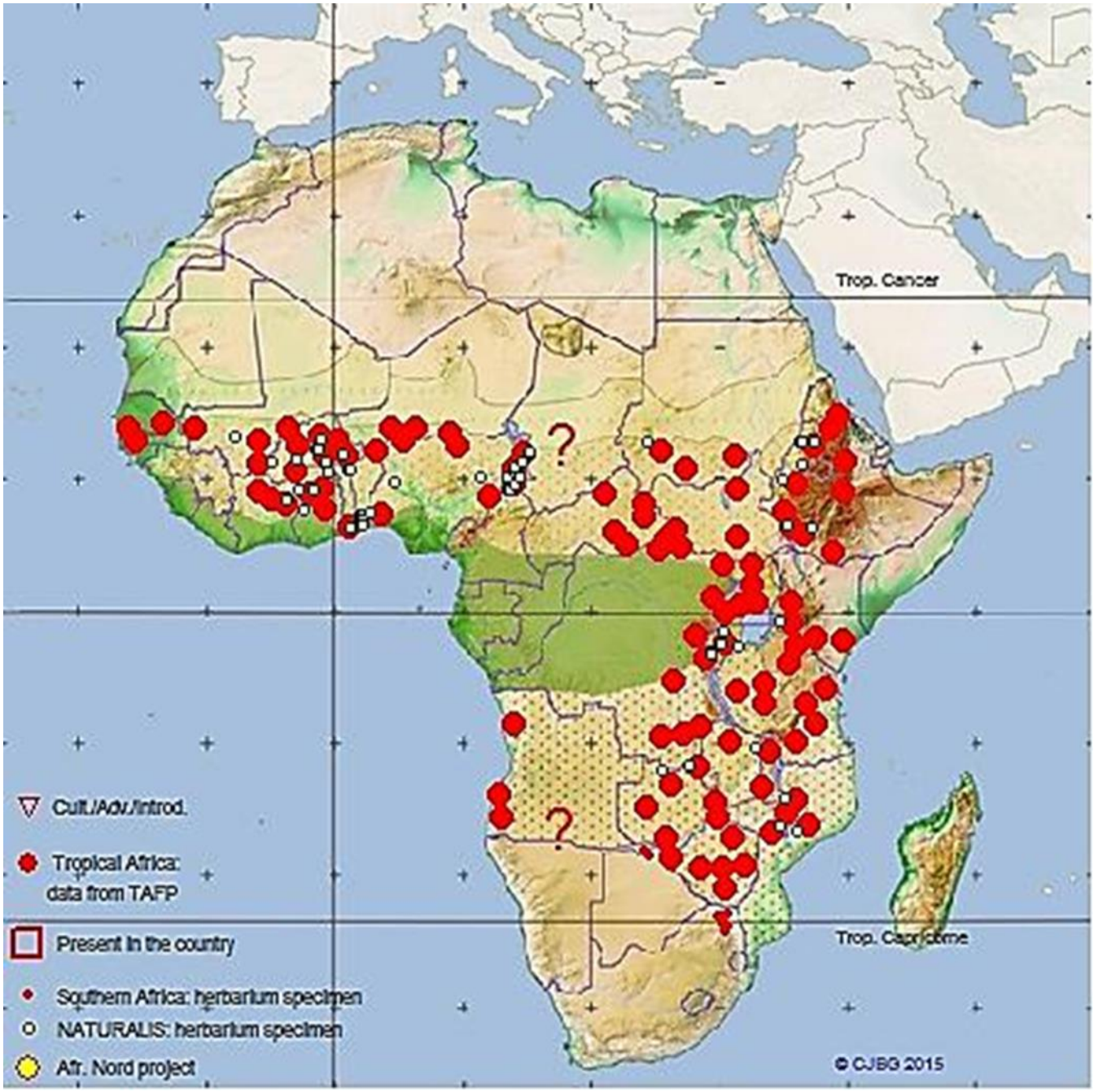

Figure (1.1) Distribution of *Acacia polyacantha* tree in the African gum belt

http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=67253

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Figure(1.2): *Acacia polyacantha* tree

((https://en.wikipedia.org/wiki/Senegalia_polyacantha))

1.7Structure of plant gums

Gum nodules contain polysaccharide material of complex nature usually contaminated with impurities such as bark fragments, entrapped dust and insects. Inert pertinacious material and a few amounts of terpenoid resins can also be present. Gums are polyuronides the uronic acid residues may carry acetyl or methyl groups and, generally, occur at least in part as methyl groups or as metallic salts. The hexose residues are present in the pyranose configuration, while the pentose residues occur in the furanose(Stephen et al.,1955 and 1957) beside the foregoing gums, *Sterculia termentosa* gum contains rhaminose, galactose and probably galacturonic acid, *Olibanum* gum was found to be of an arabino-galactan and polysaccharide containing galactose and galactouronic acid (Elkhatem et al., 1956). It was noted that the gum was very heterogeneous and it has been described as heteropoly molecular, i.e. having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer unites, in addition to distribution in molecular weight (Lewis and Smith, 1957; Dermyn, 1962 and Stoddart, 1966). According to Philips(1988) and Williams (1989), fractionation by hydrophilic affinity chromatography revealed that *Acacia Senegal* gum consists of at least three distinct components. Fraction 1 AG (arabino galactan), fraction 2AG (arabinogalactan-protein) and fraction 3GP (galactoprotein). But even those contain a range of different molecular weight components revealing the polydiverse nature of the gum. Fraction 1 containing 88% of the total has only small amount of protein content. Fraction 2 represents 10% of the total and had 12% protein content. Fraction 3 resembles 1.24% of the total but contains almost 50% of protein AGP are responsible for the emulsifying properties of gum Arabic. No mention has been made to detailed comparison between structures of gums from different species of trees, but is believed that D-galactose and uronic acid residues generally constitute the backbone of gum polysaccharide with 1- 3 and 1-6 linkages predominating side chain are characterized by presence of D-xylopyranose, L-arabinose, and L-arabino-furanose linkage (Elnour, 2007).

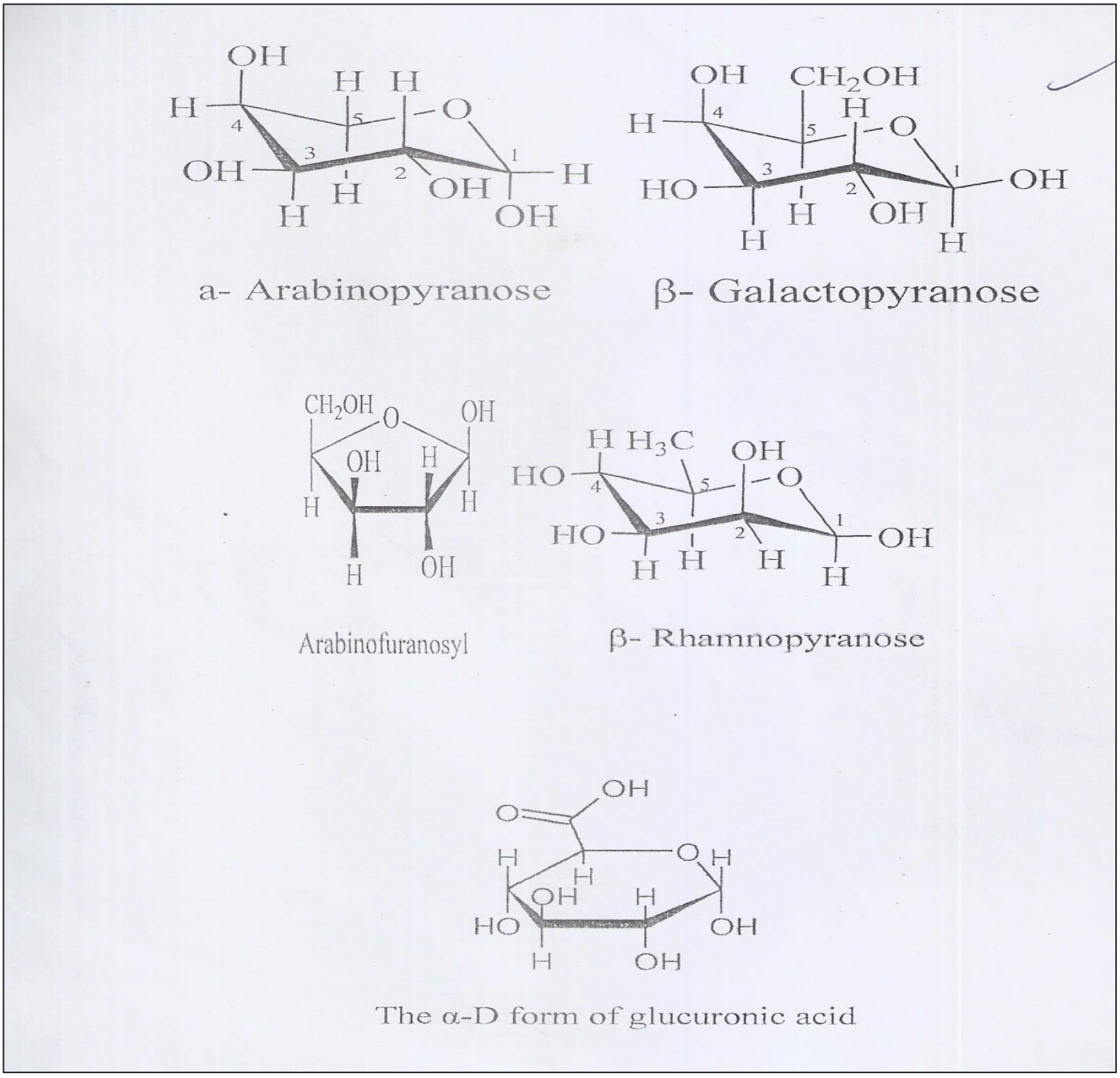
1.8 Theories of Gums formation

There are numberless of gum formation theories and functions , which have been formulated to explain the phenomena of gummosis, formation of gum exudates is pathological condition resulting from microbial (fungal and bacterial) infection of injured tree , natural factor that tends to lessen the vitality of the trees , such as poor soil , lack of moisture , and the weather improve gum yields. Other considers the production of gum to metabolic process in the plant with quantity and quality produced being function of environmental condition, some believed

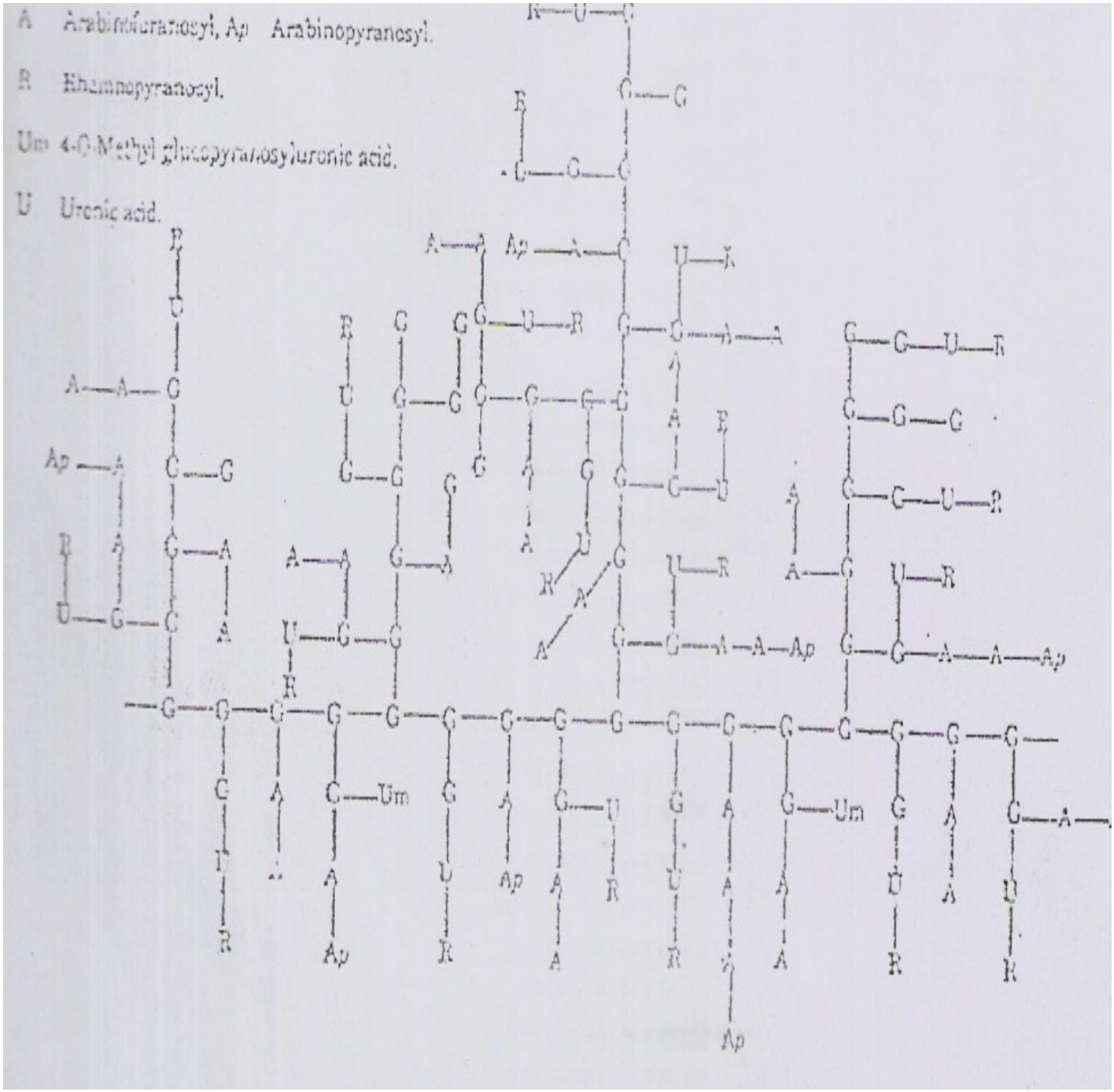
that the gum formed as a defense mechanism to seal off the wound to prevent desiccation other proposed that the starch might undergo transition into gum. The latter is refuted by Anderson and Dea 1968 , as the enzyme system necessary to transform starch into highly branched arabinoglactan with galactose , arabinose, rhamnose , glucuronic acid and its 4-o-methyl ether are complex, further(Anderson and Dea ,1968) found that the starch was not represent in tissues of excited branches and therefor proposed that gums have a hemicellulose types , highly branched arabinoglactan precursor in which rhamnose , glucronic acid and 4-o-methyl glucuronic acid are the peripheral groups(Omer, 2004).

1.9 Chemical Structure of Gums

Gum Arabic is branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salt. The backbone consists of 1,3- linked b – d-galactopyranosyl units. The side chains are composed of two to five 1,3–linked b-d-galactopyranosyl units, joined to the main chain by 1, 6-linkages. Both the main and the side chains contain units of a-1-arabinofuranosly , a-1-rhamnopyranosyle , b-d-glucuronopyranosyl, and4-O-methyl-b-d-glucuronopyranosyl, the latter two mostly as end-units(Anderson and stoddart,1966).They further analyzed the product by methylation and gel permeation chromatography and found that the uronic acid and the rhamnose residues were eliminated first which proved that they are located at the periphery of the molecule and the core was consisted of a β1,3-galactopyranose chain with branches linked through1,6 position. They also found that the protein component was associated with the high molecular weight fraction and lower molecular mass fraction was virtually exclusively polysaccharides. Figure(1.3)shows the polysaccharides in gum Arabic.(street et al.,1983) used computer modeling to analyze the previous data and proposed the structure illustrated in Figure1.4,(churms et al.,1983) subjected the gum to smith degradation leaving the reaction to reach completion after each stage of degradation procedure. They obtained different values for the composition and size of the molecule of each degradation product than those previously obtained by Anderson, 1966b, and proposed amore regular structure than the previous one proposing that the galactan core consisted of 13β-, 3-D-galactopyranosyl residues having two branches, which give single repeating sub units having molecular mass of 8×10^3 within the molecule. As the whole gum was found to have molecular weight of 560,000 thus it was proposed that the molecule consists of 64 of these subunits and that they were symmetrically arranged .(Defye and Wang, 1986) in their structural studies of gum Arabic using A25.182MHz ^{13}C-NMR (Alaa. M.M, 2015).



Figure(1.3)The Structureof carbohydrates units of gum molecule(street et al. 1983)



Figure(1.4):The Structure of *Acacia senegal* gum as proposed(street et al., 1983)

1.10 Applications of gums

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

1.10.1 Applications in the food industry

Gums for their high viscosity in solutions and inability to crystallize, are, particularly, suited to serve in foodstuff such as: thickeners for beverages, stabilizers for oil and water emulsions and as wider application where the function is to prevent agglomeration and setting of minute particles. They are designed to incorporate flavors in confectionery such as pastilles and gum drops, and the preparation of lozenges. The role of gum Arabic in confectionary products is, usually, either to prevent crystallization of sugar or to act as an emulsifier (Glickman et al.,1973).

1.10.2Pharmaceutical and cosmetic applications

Gums are used as a suspending and emulsifying or binding agents in pharmaceutical industries, it has been used in tablet manufacturing, where it functions as a binding agent or as a coating prior to sugar coating, sometimes in combination with other gums .*A. polyacantha* gum used to act as general health tonic as antidote for snake bite, and cure for venereal diseases. A preparation from the bark is used for general stomach disorders(Voget, 1995).

1.10.3 Other industrial uses

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

1.11Foam fractionation process

Foam concentration/fractionation is a separation technique in which surface-active solutes are either concentrated from a dilute solution or separated from a mixture by preferential adsorption at a gas liquid interface created by sparging an inert gas through the solution. These gas bubbles entrain the surfactant solution and form stable foam with a large gas liquid interfacial area. As the foam moves through the column, the surfactant solution tends to drain due to gravity and capillary forces. This results in a decrease in the amount of liquid in the foam. The reduction in the entrained liquid is first associated with the bubbles forming the closest spherical packing, after which they will deform to a dodecahedral shape and then possibly coalesce. Consequently, there is an increase in the gas liquid interfacial area per unit volume of the liquid. The surfactant tends to adsorb preferentially at the gas liquid interface. At the top of the column, the foam is sent to a foam breaker where the foam is broken either mechanically or chemically. This results in either enrichment or concentration of more surface-active protein because of the recovery of adsorbed protein from the gas liquid interface into the bulk entrained liquid. In the case of a dilute solution of a single protein, the extent of enrichment would depend upon the relative amount of adsorbed protein compared to that in the bulk entrained liquid. In the case of a mixture of proteins in solution, the separation of a protein from the mixture would depend upon the extent of preferential adsorption of that protein at the gas liquid interface. Since the adsorption isotherm usually leads to a much higher proportion of adsorbed protein at very low bulk concentrations, foam concentration is very effective for extremely dilute solutions. Because of the presence of hydrophilic and hydrophobic functional groups, proteins are surface active. Therefore, foam-based separations are viable for separation of protein solutions. Foam based separation has been applied to various proteins and enzymes (G. Narsimhan, 2000).

1.11.1 Performance Characteristics of foam fractionation

To evaluate the performance of the separation the following criteria are considered.
Enrichment(Ef) is defined as the ratio of foam concentration to that of initial feed.

$$Ef = \frac{\text{Concentration of protein in the foam}}{\text{Concentration of protein in the initial solution}}$$

On the other hand, the recovery of protein ratio(PR) is the fraction of feed protein recovered in the foam. It determines the efficiency of the process and is given by:

$$PR = \frac{K_d}{(K_d + V_r/V_f)_s} \times 100$$

Where K_d is the distribution coefficient, defined as the ratio of protein concentration in foam to that of the residual solution, Vr and V_f are the respective volumes of the residue and foam after separation.

The residual ratio(RR) is also considered as a measure of the residual concentration with respect to the original feed concentration:

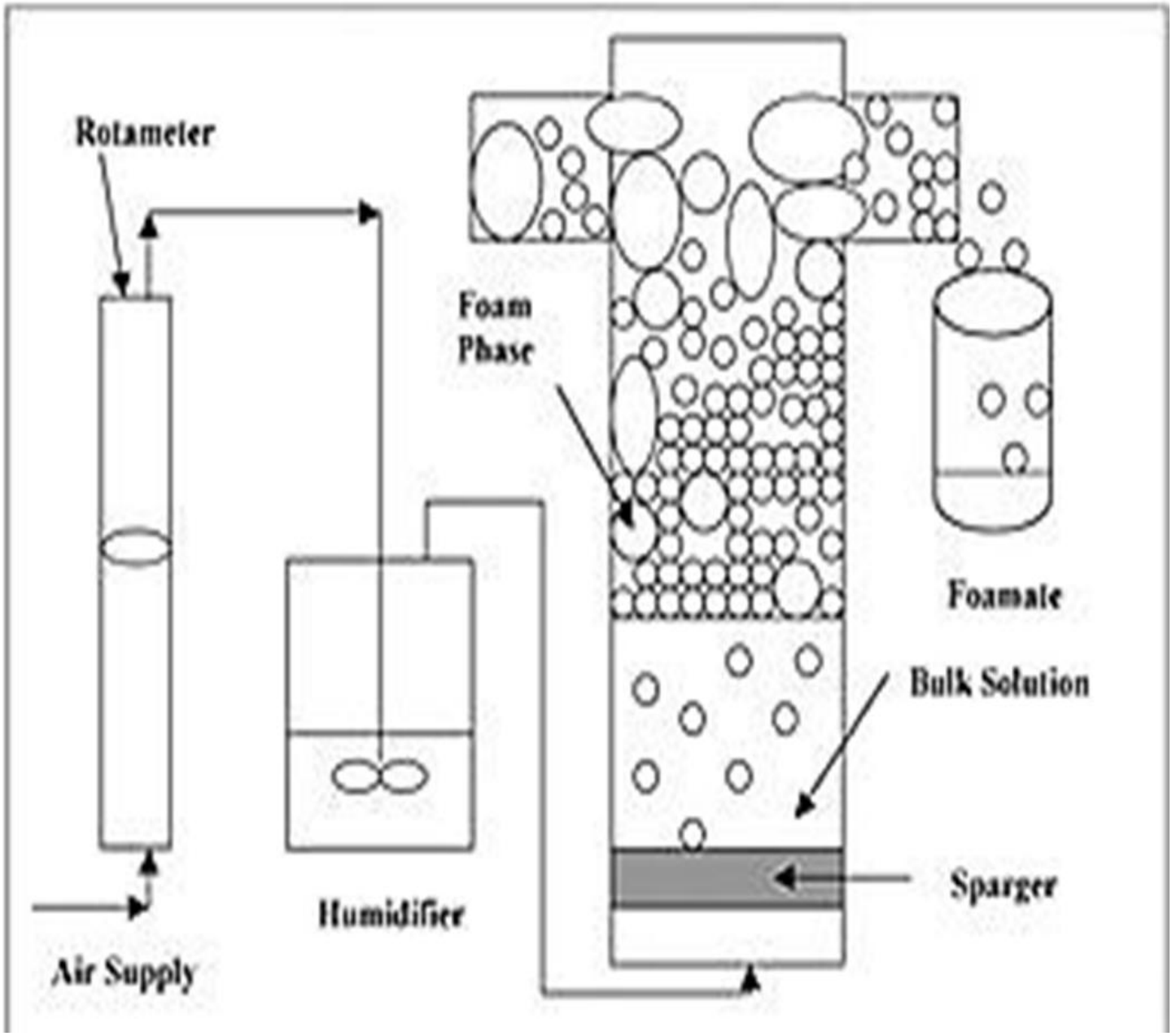
RR =Concentration of protein in the residual solution Concentration of protein in the initial solution:

$$RR = \frac{Concentration\ of\ protein\ in\ the\ residual\ solution}{Concentration\ of\ protein\ in\ the\ initial\ solution}$$

The volume of the foam produced is also a measure of the performance as this relates to the loss of liquid from the initial solution

1.11.2 Protein Denaturation at Gas–Liquid Interfaces

Protein molecules, in their native conformation, are interconnected polypeptides arranged in specific structures. Generally speaking, both hydrophobic and hydrophilic groups exist at the surface of a protein molecule. At the air-water interface, these hydrophobic groups tend to escape from the water side to spread at the interface, while the hydrophilic groups prefer to stay in the water side, Figure(1.5 and1.6).Given enough time, the tertiary conformation of the protein molecule will be changed, sometimes combined with the loss of its bioactivity if the change is irreversible(Miller et al., 1998). Such loss of bioactivity is commonly known as denaturation and can have a deleterious impact upon the functionality of the molecule because denaturation tends to be irreversible. In the industrial foam fractionation of nisin, a 10% denaturation is typical. It can be seen that, in the case of foam fractionation of proteins for example, the very method of recovering the molecules from solution can preclude their efficient subsequent use, but this tends to be overlooked. Thus, it is very important to discuss denaturation herein. Graham and Philips published an extensive study on the kinetics of the adsorption and denaturation of proteins at gas–liquid interfaces. When the protein of bovine serum albumin adsorbed, the surface pressure (the difference in the initial surface tension and the dynamic surface tension) changed simultaneously with the surface excess. However, when the globular protein Lysozyme adsorbedthe gas–liquid interface



Figure(1.5) Diagram of laboratory batch foam - fractionation apparatus. Arrows denote the direction of air flow,(***http://www.asiafoodjournal.com/2009/04/the-science-of-foams/6023/)***

Figure(1.6) Foam fractionation process

The surface pressure increased at a greatly longer time constant than the surface excess, indicating significant unfolding of protein after the surface excess had approached its equilibrium value. In addition, it was inferred that protein adsorption was diffusion controlled at early stages, but that an energy barrier for adsorption had to be overcome as the surface excess increased. Graham and Philips(1979b) showed that proteins adsorbed in multilayers but that adsorption to anything but the first layer had no effect on the surface tension. Twenty years after the work of Graham and Philips, there were several studies that investigated denaturation specifically in the foam fractionation process, but the implications of this body of work are often overlooked by workers aiming to show that foam fractionation has utility for the removal of proteins from solution. Liu et al. (1998) demonstrated the denaturation of enzymes during foam fractionation, and denaturation of the protein β-casein was detected during foam fractionation using a variety of experimental techniques, including circular dichroisim. Significantly more β-casein molecules returned to their native state upon desorption than did molecules of the enzyme pepsin. In addition, it was concluded that protein denaturation was because of conformational changes and that damage due to oxidation was negligible. Clarkson et al.(1999b) detected changes in conformational state in other proteins. Thus, it is clear that the foam fractionation of proteins and enzymes causes some denaturation, but the degree of denaturation depends on the particular molecule that is adsorbing(Paul Stevenson and Xueliang Li ,2014) .

1.12 Physical properties of gums

The solubility, color, taste, odor and viscosity are important parameters in determining the uses and commercial values of gums.

1.12.1 Solubility

Regarding to the solubility the true gums are divisible into three classes(Mantel. 1965):

(i) Soluble gums that dissolve in water forming transparent solution, e.g. *Acacia Senegal*

(ii) Insoluble gums which also absorb water but on addition of sufficient water break down into very thick transparent solutions, e.g. *tragacnth* gum.

(iii) Half- soluble gums that partially dissolve but on addition of more water pass into solution; e.g. *ghalti* gum.

1.12.2Colour

The color of the gums varies from almost colorless through various shades of yellow, orange to dark brown, some of the best gum Arabic "*Acacia senegal*" is almost colorless. On the other hand, dark or even black gums sometimes occur, e.g. *Mesquite* gum(Howes. 1949). The color of *Anogeissus leiocarpus* gum maybe yellow or light brown (Smith and Montgomery, 1959).

1.12.3 Shape

Gums collected in natural states are represented by a variety of shape and form. Usually the augments are irregularly globular or tear shaped. The grading of gum is based on shape, size and color of gum nodules.

1.12.4Viscosity

The viscosity of a liquid is its resistance to shear, to stir or to flow through a capillary tube. Since viscosity involves the size and the shape of the macromolecule it was seen as the one of the most important analytical and commercial parameter. The viscosity of the solution may be having a complicated variation with composition due to the possibility of hydrogen bonding among the solute and solvent molecules(Omer, 2004).Although gum Arabic is a high molecular weight it is a highly soluble gum, it makes a rather low viscosity, higher viscosity is not obtained with gum until the concentration of about 40-50% (Glicksman, 1969). The intrinsic viscosity of fresh collected gum varies from 14 to 60cm^3/ g and the mean viscosity changes with the age of the crop, season and samples picked from one tree (Duvallets, et al., 1993). Viscosity plays a role involving the size and the shape of the macro-molecule. It can be presented in various terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity, kinematic or dynamic viscosity and intrinsic viscosity. Anderson(1966a) in an early investigation for electro-dailized fractions of *Acacia.senegal* gum showed that the intrinsic viscosity for this gum was 20$cm^{-3}g^{-1}$. Anderson (1983) reported 13.4$cm^{-3}g^{-1}$ intrinsic viscosity for authenticated specimens and 17$cm^{-3}g^{-1}$ for commercial samples of *A.senegal* gums . Vandevelde et al.,(1985) found that the intrinsic viscosity for *A.senegal* gum originated from Sudan was in the range of 15.5 to 40cm^{-3} g^{-1}. Idris (1989) measured the intrinsic viscosity of samples obtained from *A.senegal* trees of different ages and concluded that it ranged from 7.2 to 14.2 cm^{-3} g^{-1} and that of stored samples to be 17.8 to 18.6 $cm^{-3}g^{-1}$. Anderson et al.(1991b) reported 16 $cm^{-3}g^{-1}$ for Sudanese samples Jurasek et al.(1993) surveyed the analysis of 18

specimens of *A.senegal* and found their values range from 13.4 to 23$cm^{-3}g^{-1}$. For *A.seyal* Anderson and Weiping(1991c) reported the values of 12, 14,15,17,19and21$cm^{-3}g^{-1}$intrinsic viscosity for samples from Niger, Uganda and Sudan. Hassan 2000 reported the range of 11-17$cm^{-3}g^{-1}$.The values of15.8$cm^{-3}g^{-1}$, 20.7$cm^{-3}g^{-1}$were reported for *A.polycantha* and *A.laeta* respectively(Anderson,1978&Karamalla,1965). Siddig(1996) analyzed about 94*A.senegal* gum samples for intrinsic viscosity and she reported the mean value of16.44 $cm^{-3}g^{-1}$(Siddig. N. E.,2003).

1.12.5 Specific optical rotation

The optical activity of organic molecules (saccharide and carbohydrates) is related to their structure and its characteristic property of the substance. The gum is of natural Origen, e.g. *A. Senegal* gum has the property of rotating the polarized plane of light. The direction of the rotation, as well as the magnitude is regarded as a diagnostic parameter to establish that gum used to conform to current regulatory position. *Acacia Senegal* gum gives a negative optical rotation ranging between -27 to -34 the optical rotation is used to differentiate between A. *Senegal* gum and other botanically related *Acacia* gum.(Anderson and Stoddard, 1966reported that the specific rotation for electrostatics *Acacia Senegal* gum as -31.5. Pure gum from *Acacia Senegal* has specific rotation of -27 to -30 certain variation in the degree of the optical rotation (-27 to -32) has been noticed by Anderson1968, karamalla 1998 found that the mean of the specific optical rotation of commercial *Senegal* gum was(-30.54). The optical rotation is not affected by both auto hydrolysis and variation, while mild acidic hydrolysis has a significant effect on optical rotation (Barron, 1991). Omer(2004) reported that the mean of the specific rotation of authenticated samples of A *polyacantha* gum was -16.6(Elnour, 2007).

1.13 Chemical properties of gums

1.13.1 Moisture content

Water content or moisture content is the quantity of water contained in a material, such as soil, rock, ceramics, fruit, or wood. Water content is utilized in a wide range of scientific and technical areas, and is expressed as a ratio, which can range from 0(completely dry) to the value of the materials' porosity at saturation. Moisture content of the gum identifies the hardness of the gum and hence the variability of densities and the amount of air entrapped during nodule formation. The moisture content is weight loss due to the evaporation of water(Person, 1970). It shows the hardness of the gum and hence variability of densities, the amount of densities, and the amount of air entrapped during formation.

Omer(2004) recently, reported that the moisture content of *A.polyacantha* gum to be around 8.2%.

1.13.2Total ash content

Ash is the inorganic residue remaining after the water and organic matter has been removed by heating in the presence of oxidizing agents, which provide a measure of the total amount of minerals within food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the analyte) can be distinguished from all the other components (the matrix) within gum in some measurable way. Previously ash content for *A. polyacantha* gum was determined as 2.929 ash% (Anderson, 1985).

1.13.3Nitrogen and protein content

Nitrogen is present in all organisms, primarily in amino acids (and thus proteins), in the nucleic acids(DNA and RNA) and in the energy transfer molecule adenosine triphosphate. The human body contains about 3% by mass of nitrogen, the fourth most abundant element in the body after oxygen, carbon, and hydrogen. The nitrogen cycle describes the movement of the element from the air, into the biosphere and organic compounds, then back into the atmosphere. The role of nitrogen and nitrogenous component in the structure, physico- chemical properties and functionality of gum Arabic was subjected to intensive investigation(Anderson et al., 1985).On the other hand Erick Dickinson(1991) studied the emulsifying behavior of gum Arabic and concluded that there was a strong correlation between the proportion of protein in the gum and its emulsifying stability, Siddig(1996) reported that the average value of nitrogen content of *Acacia senegal* gum and 80% of authenticated samples analyzed were in the range(0.27-0.39%). Omer(2004) reported that the mean of nitrogen content of *A. polayacantha* gum samples was 0.35%.

1.13.4Equivalent Weight and Uronic Anhydride

Titrable acidity, which is the numbers of mills of 0.02N sodium hydroxide that neutralizes 10 ml of 3% gum solution, represented the acid equivalent weight of gum. From which the uronic acid content, could be determined (Karamalla, 1965; Anderson et.al, 1983;

Vandevelde, 1985). Gums were found to differ widely in their equivalent weight and uronic acid content (Karamalla. 1965).

1.13.5Molecular Weight

The molecular weight of the polymers can be identified from physical measurement or by application of chemical methods. Applications of chemical methods require that the structure of the polymer should contain well known number of functional groups per molecule and they invariably occur as end groups. The end group analysis method gives an approximate number of molecules in a given weight of sample; they yield the average number of molecules for polymeric materials. This method becomes insensitive at high molecular weight, as the fraction of end groups becomes too small to measure with precision. This is explained by the fact that fraudulent sources of the end groups not considered in the assumed reaction mechanism steadily become consequential as the molecular weight increases and the number of end groups diminishes to such an extent their quantities determination is not feasible. Those reactions confine frequent application of chemical methods to condensation polymers with average molecular weight seldom exceeding 2.5×10^3(Flory,1953).The molecular weight of gums varies greatly in values due to gum heterogeneity as well as a variation in the techniques used to separate, purify and determine the molecular weight. These methods include: Ultracentrifugation, Molecular sieve chromatography, osmometry, intrinsic viscosity, measurements gel filtration and light scattering. GPC be coupled on line to multi angle laser light scattering.(MALLS)has been demonstrated to be a very powerful method for characterizing highly polydisperse polymer systems and the molecular weight of A.*senegal* gum was found to be equivalent to 5.4×10^5(Picton, 2000). The weight average molecular weight for A.*polyacantha* gum using the GPC-MALLS was reported to range between 2.94×10^5 to 7.346×10^5(Omer ,2004).

1.13.6 Acidity and pH Measurements

The hydrogen ion concentration plays great importance in the chemistry and industry of the gums. The change in the concentration of hydrogen ion may determine the solubility of gum and the precipitation of protein. Therefore, functional properties of gum may be affected by change in pH for example viscosity and emulsifying power. Crude gum is slightly acidic because of the presence of few free carboxyl groups of its constituent acidic residues, D-glucuronicacid and its 4-O-methy1 derivatives. Karmallah et al.,(1998)

reported the pH mean value of 4.66 for the 755authentic *A.senegal var.senegal* gum samples, collected in season 1994/1995, also they reported an average value of 4.4 for *A.senegalvar.senegal* gum samples(Omer.A.A, 2015)

1.13.7 Osmotic pressure

Osmometry:

Based on the phenomenon of osmosis, the membrane osmometry is widely used technique for number average molecular weight(M_n) determination. Osmotic pressure is the only one of the four colligative properties that provides a convenient practical method for measuring Mw. It can be used to identify molecular weight for macromolecules. In osmosis, the semi permeable membrane allows the solvent molecules to pass freely but the polymer molecules are unable to pass through it. Thus, when a pure solvent is separated from the polymer solution by a semi-permeable membrane then there is a net flow of solvent towards the cell containing the polymer solution. This causes an increase in liquidity in the polymer solution cell which then subsequently produces a rise in liquid level in the corresponding measuring capillary tube. The rise in the liquid levels opposed and balanced by a hydrostatic pressure which results due to the difference in the liquid levels of the two measuring capillary tubes. This difference in the liquid levels is directly related to the osmotic pressure of the containing polymer solution. Thus, static equilibrium is achieved when no further flow of solvent towards solution side occurs. The difference in height (h) of the liquids in the tubes may be converted to osmotic pressure (π).

$$\pi = h.d.g$$

Where(g) is the gravity, d is the density of solution. The osmotic pressure of the polymer solution is related to M_n by Vant Hoff's equation.

$$\pi = C\ R\ T$$

$$\pi = (RT/M_n)\ C$$

$$\pi/C = RT/M_n$$

A plot of π/C VsC gives straight line.

The intercept of which is RT/M_n, when the curve is extrapolated to zero concentration, M_n can be calculated from the value of the intercept. The vant hoff's equation does not apply to polymer solution, even though they are very dilute. The concentration related by osmotic pressure is expressed by complex equation.

$$\pi = RT(A1\ C + A2\ C2 + A3\ C3 + \dots)$$

$$\pi/C = RT(A1 + A2\ C + A3\ C2 + \dots)$$

Where:

A1,A2, A3 are first, second and third virial coefficient. C is concentration of polymer in solution. The first virial co-efficient is related directly to molecular mass by relation $A=1/M_n$(Tager, 1978). Then the equation becomes

$$\pi C = RT(1/M_n + A2\ C + A3\ C2 + \dots)$$

$$\pi C = RT/M_n\ (1 + \Gamma 2\ C + g\ \Gamma 2\ C2 + \dots)$$

Where: $\Gamma = A2/A1$ and g is varying function of the polymer solvent interaction (equal zero for poor solvent. 0.25 for good solvent) C2 may be neglected then the equation becomes

$$\pi C = RT/M_n\ (1 + \Gamma 2C)^2$$

$$\text{Or } (\pi C)^{1/2} = (RT/M_n)^{1/2}\ (1 + \Gamma 2C)^2$$

When plot $(\pi C)^{1/2}$ Vs C that gives a straight line, the molecular mass can obtain from intercept, and the second virial co efficient from slope(Alaa, 2015).

$$\text{Intercept} = (RT/M_n)^{1/2} \quad \text{Slope} = (RT/M_n)^{1/2}\ A_2 M_n/2$$

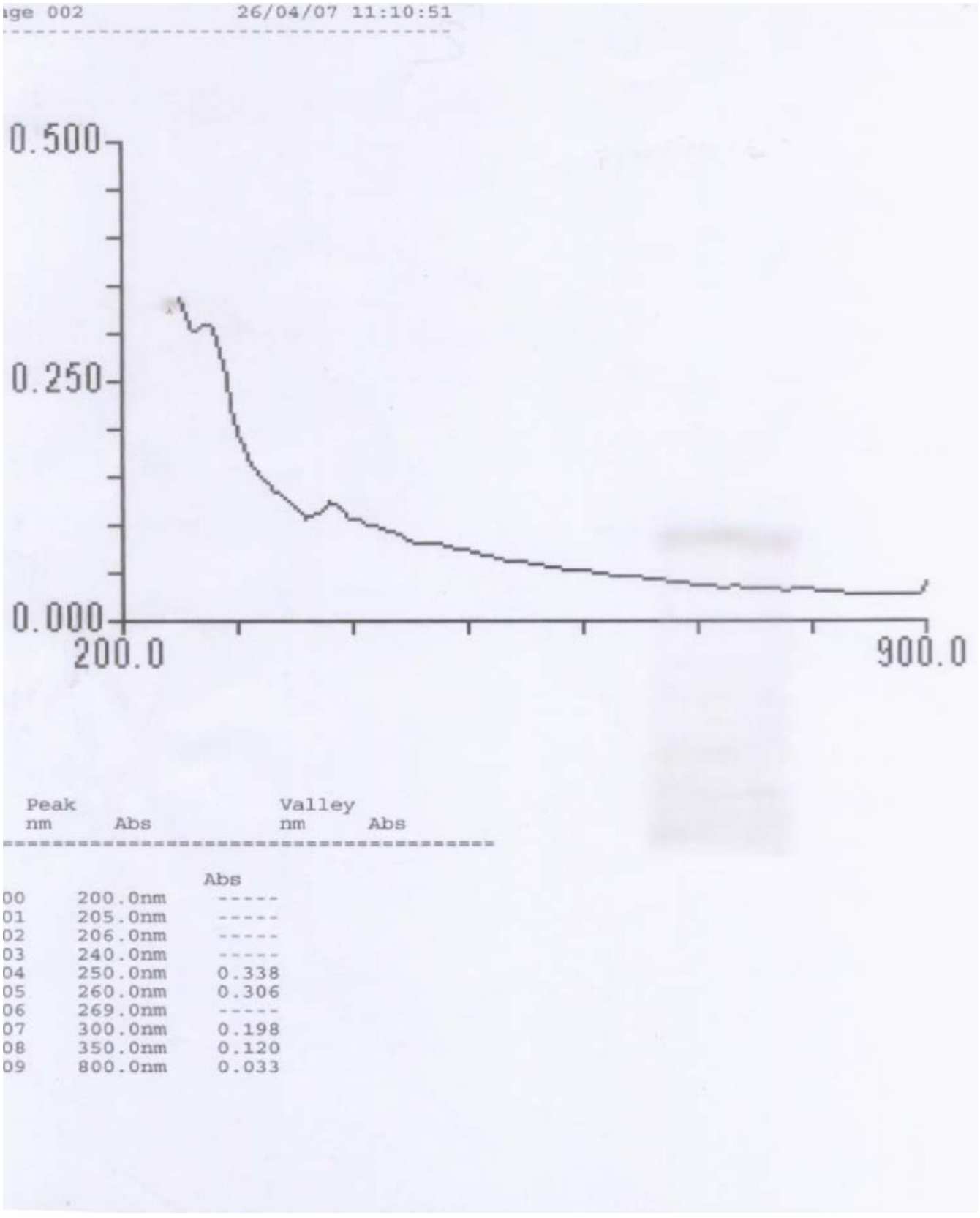
1.13.8 Cationic composition

Atomic absorption is a technique which can be implemented effectively to determine about 70 elements. It is based on the absorption of radiations by the atoms of a particular element in the ground state, raising them to excite states. Excitation is produced by radiation energy at a wavelength equivalent to the energy needed to lift an atom from its ground state to higher level, the energizing radiation is thus absorbed and the amount of absorption is directly dependent on the population of the ground state atoms in the flame.

The sample solution is aspirated in the gaseous state by vaporization and dissociation of molecules. A hollow cathode lamp, which consists of a cathode of the element of interest or coated with it and anode at a low pressure of neon or argon, is used as a source of radiation. A monochromatic is utilized in conjunction with the hollow cathode lamp to isolate the desired spectrum. The radiation that finally reaches the detector system is amplified.(Omer .A.A, 2015)

1.13.9 Ultraviolet(UV)

Ultra violet is not used primarily to show the presence of individual groups, but rather to the relationship between functional groups chiefly conjugated either between carbon or carbon oxygen double bonds, between double bonds, and in aromatic ring and even in the presence of aromatic ring itself. It can additionally reveal the number and location of constituents attached to the carbons of the conjugated system(Boyd, 1978) Figure(1.7).



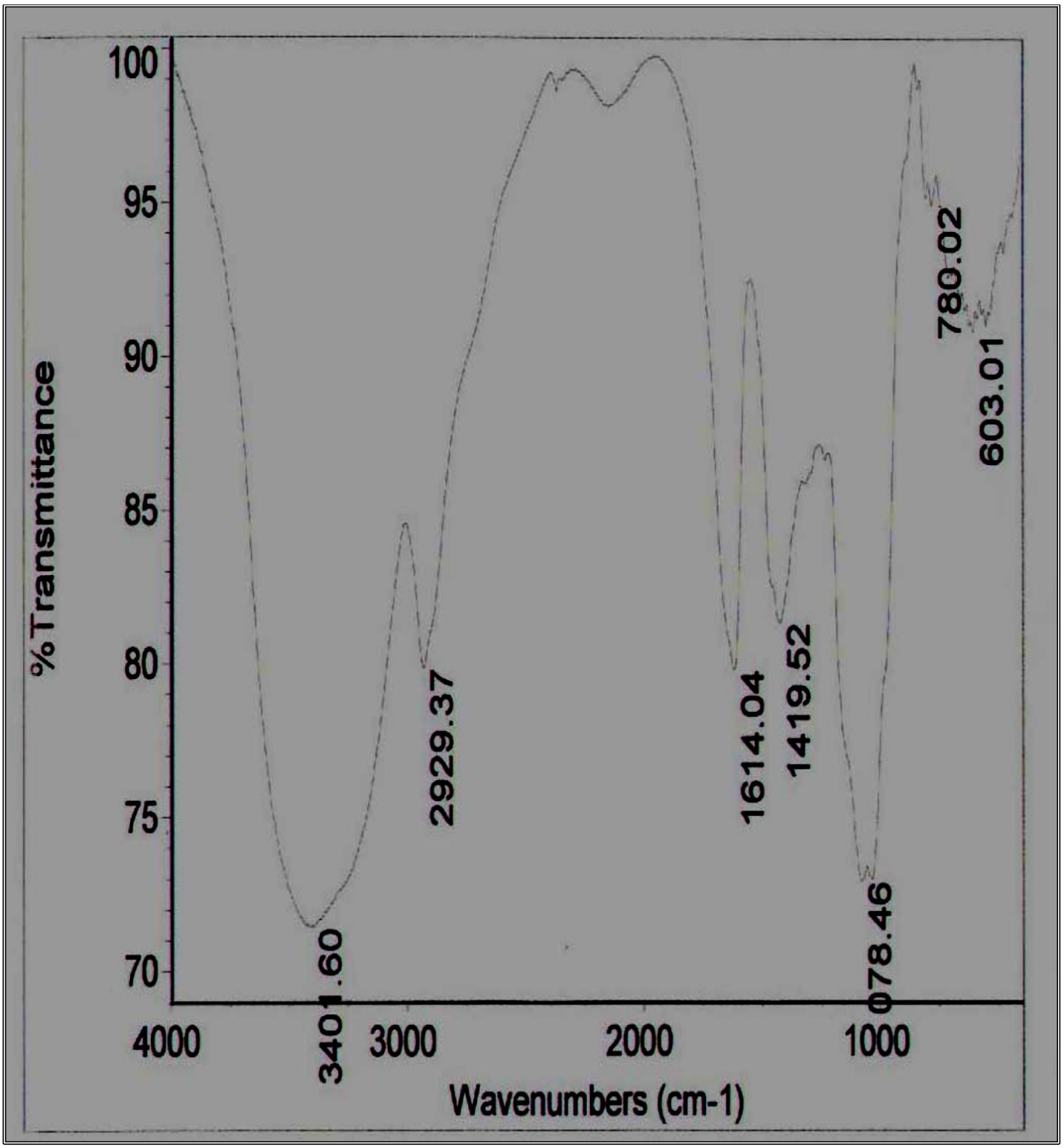
Figure(1.7) UV absorption spectra of fraction 2 of *A. polyacantha* gum(Elnour ,2007)

1.13.10 Infra-red spectroscopy (IR)

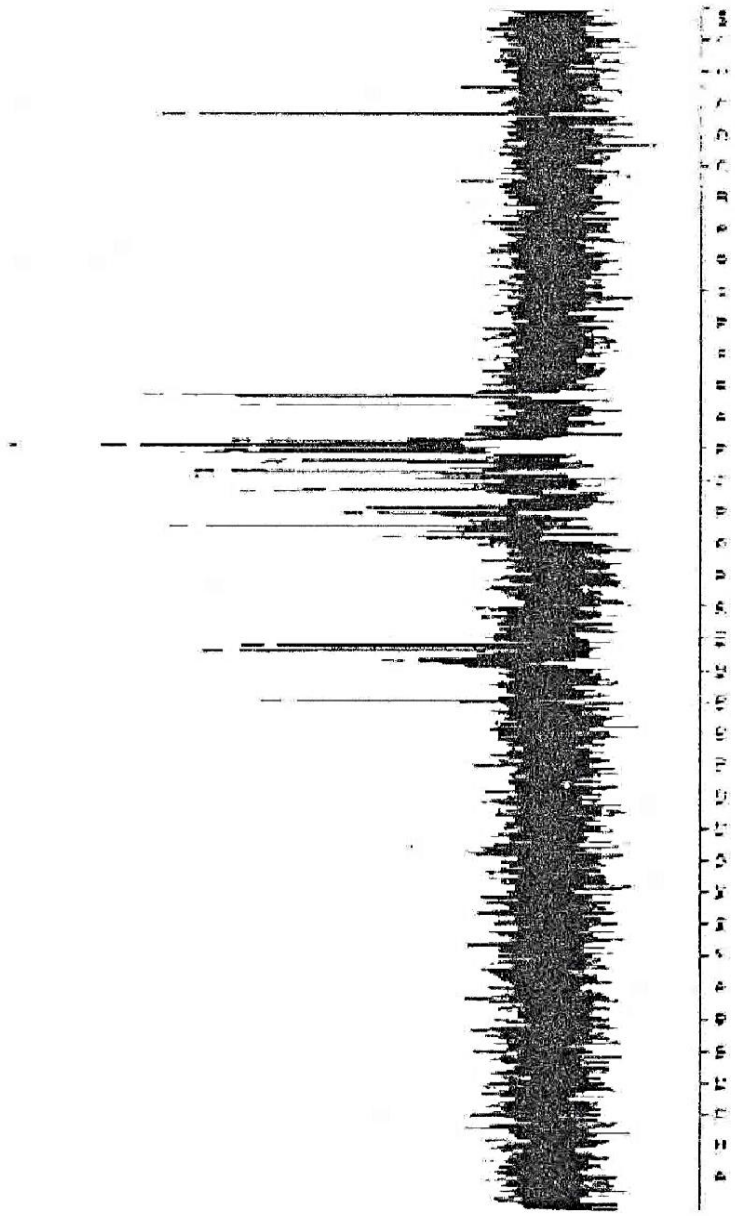
Infrared spectrophotometry is one of the most powerful tools available to identifying pure organic and inorganic compounds because each molecular species has a unique infrared absorption spectrum. Thus, an exact match between the spectrum of a compound of known structure and that of an analyte unambiguously identifies the latter (Daly et al., 1990). FT-IR spectrometric measurements for gum of *Acacia Senegal* from Gum Arabic Company Ltd Elobied branch showed eight peaks with abroad one at about 3445.65 cm^{-1} most likely for hydroxyl groups(OH), and two at 1700-1600 cm^{-1} probably for carboxyl, aldehyde or ketone groups Fig(1.8), the remaining peaks were specific for the gum sample.

1.13.11 ^{13}CNMR Spectroscopy

The gum polysaccharide from *Acacia polyacantha* when subjected to CNMR (B2O, 50mg,Top: dept-135, Bottom: ^{13}C) analysis, gave a well resolved B.n.m.r spectrum, many Signals at different chemical shifts and intensities were observed indicating the presence of Carbon atoms in different environments. However, the major characteristic chemical Shifts given by the *polyacantha* gum are shown on the Figure1.9 (Omer, 2014).



Figure(1.8) FT.IR Spectrum of *Acacia senegal* collected from Gum Arabic Company Ltd Elobied branch(Mohammed, 2006)



Figure(1.9) ^{13}CNMR Spectrogram of *Acacia polyacantha* gum(Omer ,2014)

1.13.12 Objectives

The objectives of this study are:

1.Fractionation of *Acacia polyacantha* gum into two fractions, hydrophobic and hydrophilic fractions using a foaming method

2. Studying of physicochemical properties of the fractions

3.Comparison of the results, which obtained with the results of some previous studies of crude *Acacia polyacantha* gum.

Chapter two

Materials and Methods

Chapter two

Materials and Methods

2.1 Materials

The samples of *Acacia Polyacantha* gum were collected from Eldamazine (Blue Nile State) during the season 2012.Then were cleaned by hand to ensure they were relatively free from sand, dust and bark impurities , coning and quartering . Then were grounded using pestle and mortar, backaged in labeled self-sealing polyethylene bags for analysis.

- Hydrochloric acid (50%)
- Sulphuric acid (conc.)
- Sodium hydroxide (30%w/v)
- Nitric acid (33%)
- Kjeldhal tablet(copper sulphate-potassium sulphate catalyst)
- Boric acid (2%)
- Methyl red indicator
- Distilled water

Fractionation of *polyacantha* gum solution:

It was done according to Abdel Rahim(2006). Gum solution(12.7%) was prepared in distilled water and the solution was then blown with air using an aerating pump till foaming stops. The foam was collected on a Petri dish and exposed to air to dry. The foam is hydrophobic Fraction(HO).The Draining was dried using freeze drying, which is the Hydrophilic fraction(HI).

Figure(2.1) Shape and colour of *Acacia polyacantha* gum and its fractions

2.2 Methods of characterization

Physicochemical characterization methods were used to determine physical and chemical properties of *Acacia polyacantha* gum and its fractions such as moisture content, ash content, total nitrogen and protein content, the specific optical rotation, pH value, refractive index, intrinsic viscosity, number average molecular weight and cationic composition.

2.2.1 Moisture content

The determination was carried out according to AOAC(1990). Crucibles were dried in Hearus oven at 105^{o}C for 30 minutes, cooled in a desiccator and then weighed(M1). About two grams of the sample were placed in acrucible and weighed accurately(M2). Contents were kept in an oven for 5 hours at 105^{o}C cooled in desiccator and re weighed(M3). Loss percentage, on drying was calculated as follows:

$$x = \frac{(M2 - M3)}{(\mathrm{M2} - \mathrm{M1})} * 100$$

M1: Weight of the empty crucible

M2: Weight of crucible +sample

M3: Weight of crucible +sample after drying

2.2.2Ash content:

Crucibles were heated in an oven for 30 minutes cooled in a desiccator and then weighed(W1). About two grams of the sample were placed in the crucible and accurately weighed (W2), then ignited at 550^{o}C in a furnace for 2 hours, cooled in a desiccators and weighed(W3). Total ash% was calculated as follows:

$$\text{Total ash } \% = \frac{\mathrm{W3}-\mathrm{W1}}{\mathrm{W2}-\mathrm{W1}} * 100$$

W1: Weight of the empty crucible(g)

W2: Weight of crucible +sample(g)

W3: Weight of crucible +sample after drying(g)

2.2.3 Specific optical rotation

1 g/100cm^{-3} aqueous solution of the gum was prepared. The solution was filtered to be highly pure. Optical rotation was measured using a(1 dm=10cm) tube filled with the test solution, at room temperature specific optical rotation was calculated.

Specific rotation =α X 100/C X L=……dm^{-1} mL g^{-1}

Where:

α = observed optical rotation
C = concentration of the solution (g/ml)
L = length of the Polari meter tube(dm)

2.2.4 Refractive index

1 g/100ml of fraction solution was used to measure the refractive index by instrument [Type: Abbe 60refractometer] at room temperature 25.C

2.2.5Total nitrogen and protein content(Kjeldal method)

0.5 gram of sample (in duplicate) was weighed and transferred to Kjeldahl digestion flasks and Kjeldahl tablet (copper sulphate-potassium sulphate catalyst) was added. 10 cm^3 concentrated sulphuric acid was added. The tube was then mounted in 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 color was observed which indicates the completion of the digestion. The tubes were then allowed to cool to room temperature. Their contents were quantitatively transferred to kjeldahl distillation apparatus followed by addition of distilled water and 30%(w/v) sodium hydroxide. Steam was then started and the released ammonia was absorbed in 25 cm^3 of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.02M, hydrochloric acid using methyl red as an indicator. Blank titration was carried in the same way.

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

Where:
M1: mills of HCl that neutralized the sample distillate
M2: mls of HCl that neutralized the blank distillate
M: molarity of HCl titrate(0.02M).

S : Sample weight(0.5g).
Protein content was calculated using nitrogen conversion factor (NCF) of 6.6 (Anderson,1986) resulting from amino acid analysis as follows:
% protein = % N x 6.6

The following equations show degradation of sample, liberation of ammonia, capture of ammonia and back titration respectively:

$$Sample + H_2SO_4 \rightarrow (NH_4)_2SO_{4(aq)} + CO_{2(g)} + SO_{2(g)} + H_2O_{(g)}$$

$$(NH_4)_2SO_{4(aq)} + 2NaOH \rightarrow Na_2SO_{4(aq)} + 2H_2O + 2NH_{3(g)}$$

$$B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$$

$$B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_{3(aq)} + NaB(OH)_{4(aq)} + CO_2(g) + H_2O$$

2.2.6Intrinsic viscosity

Viscosity was measured using U- tube viscometer for 1% aqueous solution of sample at room temperature (25 ^oC). The relative viscosity was then calculated using the following equation

Relative viscosity : $\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o}$

η : solution viscosity
η_o: solvent viscosity
t : flow time of solution
t_o: flow time of solvent

Specific viscosity : $\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \frac{t - t_o}{t_o} = \eta_{rel} - 1$

Reduced viscosity : $\eta_{rel} = \frac{\eta_{sp}}{c} = \frac{\eta_{rel} - 1}{c}$

Inherent viscosity : $\eta_{inh} = \frac{\ln \eta_{rel}}{c}$

Intrinsic viscosity : $[\eta] = \left(\frac{\eta_{sp}}{c}\right)_{c=o} = (\eta_{inh})C = 0$

The reduced viscosity was determined for Different concentration of hydrophobic fraction solution 0.5, 1, 1.5, 2, 2.5, 3g /ml and hydrophilic fraction 0.05, 0.1, 0.15, 0.2, 0.25 g/ml and was then calculated from the above equation. The intrinsic viscosity was determined by extrapolation of reduced viscosity against concentrations back to zero concentration. The interception on Y-axis gives the intrinsic viscosity.

2.2.7 Cationic composition

Accurately weighed 2 grams of dry sample were ignited to ash in a muffle furnace at 550^{o}C for 4 hours. Ten ml of 50% HCl and 5 ml of 33% HNO_3 were added to each crucible and allowed to warm for one hour to dissolve the minerals and cooled. 10 ml of HCl and 10 ml distilled water were added and allowed to stand for 15-20 minutes. The mixture was filtered with ashless filter paper No.41mm and distilled water was added to complete the volume to 100 ml in a volumetric flask. Sodium (Na^+), Calcium(Ca^+) and potassium(K^+) was determined using flame photometer. While iron(Fe^+) and magnesium(Mg^+), were determined using Atomic Absorption Spectrometer.

2.2.8PH of the gum solution

The pH was determined using a pH meter (Model 1100). This was done by shaking1% w/v dispersion of each of the sample in water for 5 min. Two standard buffer solutions of pH 4.00 and 7.00 were used for the calibration of the pH meter. The pH meter was placed at room temperature and the electrode immersed into the solution. The reading of the meter was then recorded for triplicate measurements.

2.2.9 Number average molecular weight of gum by Osmotic pressure

Different concentration of hydrophobic fraction solution 1,1.5,2,2.5,3,3.5,4, 4.5,5g/ml and hydrophilic fraction 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 g/ml were prepared by dissolving in distilled water to the mark of the flask. Each of them was filtered through filter paper. Osmotic pressure was measured using Osmomat 050(Colloid osmometer) at 25°C.

2.2.10UV Absorption spectra

Maximum, absorption spectra of (25.9mg/25ml) hydrophobic fraction and (26.1mg/25 ml) hydrophilic fraction solutions were determined using UV-VIS Recording Spectrophotometer, MODEL UV-2201, SHIMADZU.

2.2.11FT-IR spectroscopy

Twenty five mg of gum samples were mixed with 75mg KBr and then pressed in the disc to form proper kits. The kits were then transferred to the measuring compartment and subjected to Fourier Transform infrared (FT-IR) using Thermo-Nicoblet-IR300 spectrophotometer.

Chapter three

Results and Discussion

Chapter three

Results and Discussion

1.1 Physical and Chemical properties

Table(3.1) shows the physicochemical properties of crude gum and its fractions:

The moisture content for both hydrophobic and hydrophilic fractions were found to be (10.29 and 8.4%) respectively. The mean values reported here were slightly higher than the mean value of 8.2% reported by Omer (2004) for crude *polyacantha* gum.

Ash content was found to be 2.85% for hydrophobic fraction and 3.41 % for hydrophilic fraction, Results obtained were within the range of ash content of crude *polyacantha* gum (1.9 to 3.7%) which reported by Omer(2004) and range(3.06-3.67%) reported by Siddig(1996) for *A. sensgal* gum.

The nitrogen and protein contents for hydrophobic fraction were 0.56 and 3.6 % respectively, which are greater than of that in hydrophilic fraction 0.35 and 3.6% respectively. While for *Acacia polyacantha* gum were 0.35 and 2.28 % which reported by Omer (2004). The values in hydrophobic fraction is the higher than crude gum and hydrophilic fraction . Nitrogen and protein values in Table(3.1) are in a good agreement with specification for identify and purity of gum Arabic of FAO and with the values obtained by Karamalla et al. , 1998.

Aqueous solutions of all samples were found to be optically active (levorotatory). The specific rotation of hydrophobic fraction (-24º) and for hydrophilic fraction (-18º). These findings were within the range of Omer (2004) who reported -13.5º to -26.0º specific rotation with average (-17.3º) for *A. polyacantha* gum, but were higher than the values of -10.3º and -7º to -13º given by Siddig(2003), respectively.

Refractive indices of both hydrophobic and hydrophilic fractions were found to be identical and having a value of 1.685. Omer(2004) and Elkhatim(2001) reported fairly similar values of 1.3337 and 1.3339, respectively.
The pH of hydrophilic aqueous solution was found to be (3.1) which is highly acidic than the hydrophobic fraction(4.65). The PH of hydrophobic fraction was within the range of(4.6 to 5.2) which reported by Omer(2004)and it is lower than (5.23) which reported by Siddig(2003).

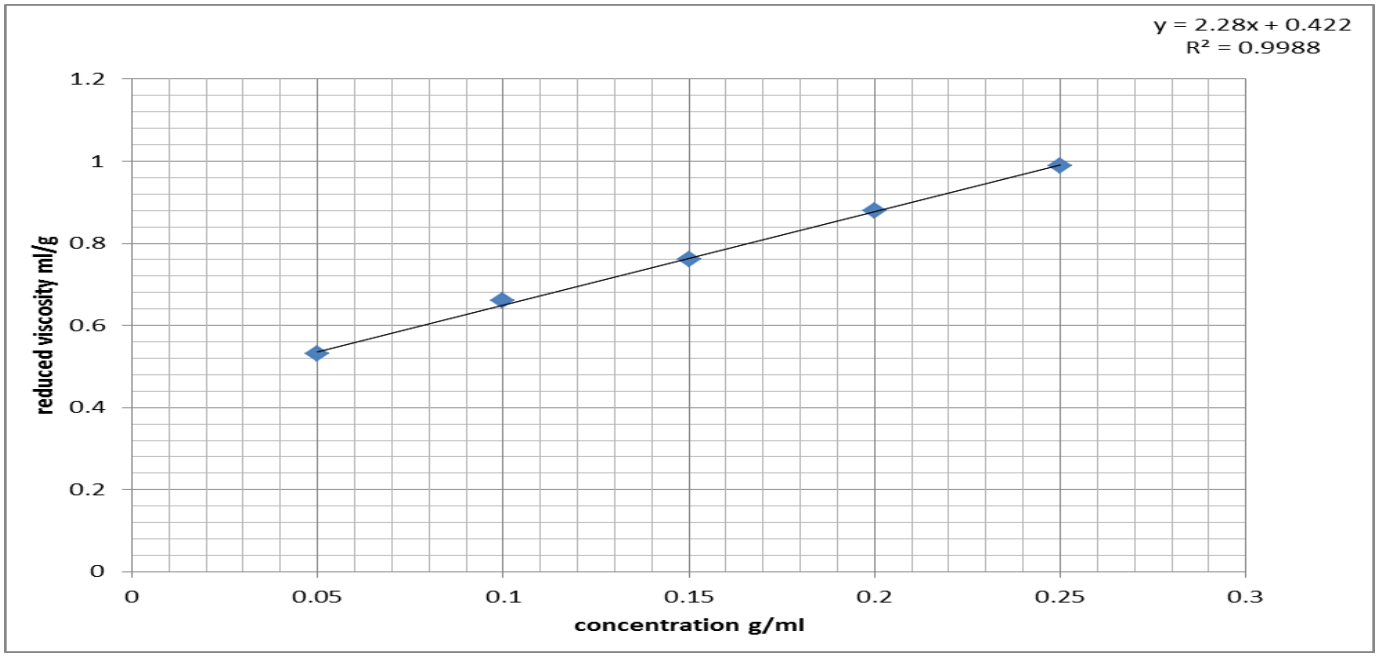
Table (3.1) physiochemical properties of *Acacia polyacantha* gum and its fractions

Samples / Parameters	Hydrophobic fraction	Hydrophilic fraction	Crude gum*
Moisture content(%)	10.29	8.4	7.5
Ash content(%)	2.85	3.41	3
Specific rotation	-24º	-18º	-17.3º
Refractive index	1.685	1.685	1.337
Nitrogen content(%)	0.56	0.35	0.35
Protein content(%)	3.6	2.31	2.28
PH value	4.65	3.1	4.9
Intrinsic viscosity	0.7807 ml/g	0.422 ml/g	10.3 ml/g
Number average molecular weight	76,310 g/mole	148,274 g/mole	136,900 g/mole

*Omer (2004)

Aqueous solutions of samples of two fractions of *A. Polyacantha* gum were showed low viscosity. As presented in Table(3.1) the intrinsic viscosity of hydrophobic fraction was 0.7807$cm^{-3}g^{-1}$, Figure(3.2) and for hydrophilic fraction was 0.422 $cm^{-3}g^{-1}$, Fig(3.1), while the intrinsic viscosity of crude *Acacia polyacantha* gum sample showed the value of 10.3 $cm^{-3}g^{-1}$reported by Omer (2004), however Siddig (2003) recorded slightly higher value of 12.7$cm^{-3}g^{-1}$.These values were markedly lower compared to the 14 $cm^{-3}g^{-1}$intrinsic viscosity reported for *A. seyal* gum (Elkhatim, 2001)

The number average molecular weights of hydrophilic and hydrophobic fractions were calculated from the intercept of the graphs of Figures (3.3 and 3.4). The values of the number average molecular weight obtained from the osmotic pressure measurements. It is clear from Table(3.1) that the mean value of number average molecular weight of hydrophobic fraction is(76,310g/mole) which is less than value of hydrophilic fraction(148,274 g/mole), while the number average molecular weight hydrophilic fraction is in a close agreement to the mean value of (136,900 g/mole) for *Acacia polyacantha* gum reported by Omer(2004) , but it was higher if compared to the range($3.02X10^3$ to $3.90X10^3$) of *Acacia polyacantha* gum reported by Elnour 2007.



Figure(3.1) A plot of concentrations as a function of reduced viscosity for hydrophilic fraction samples

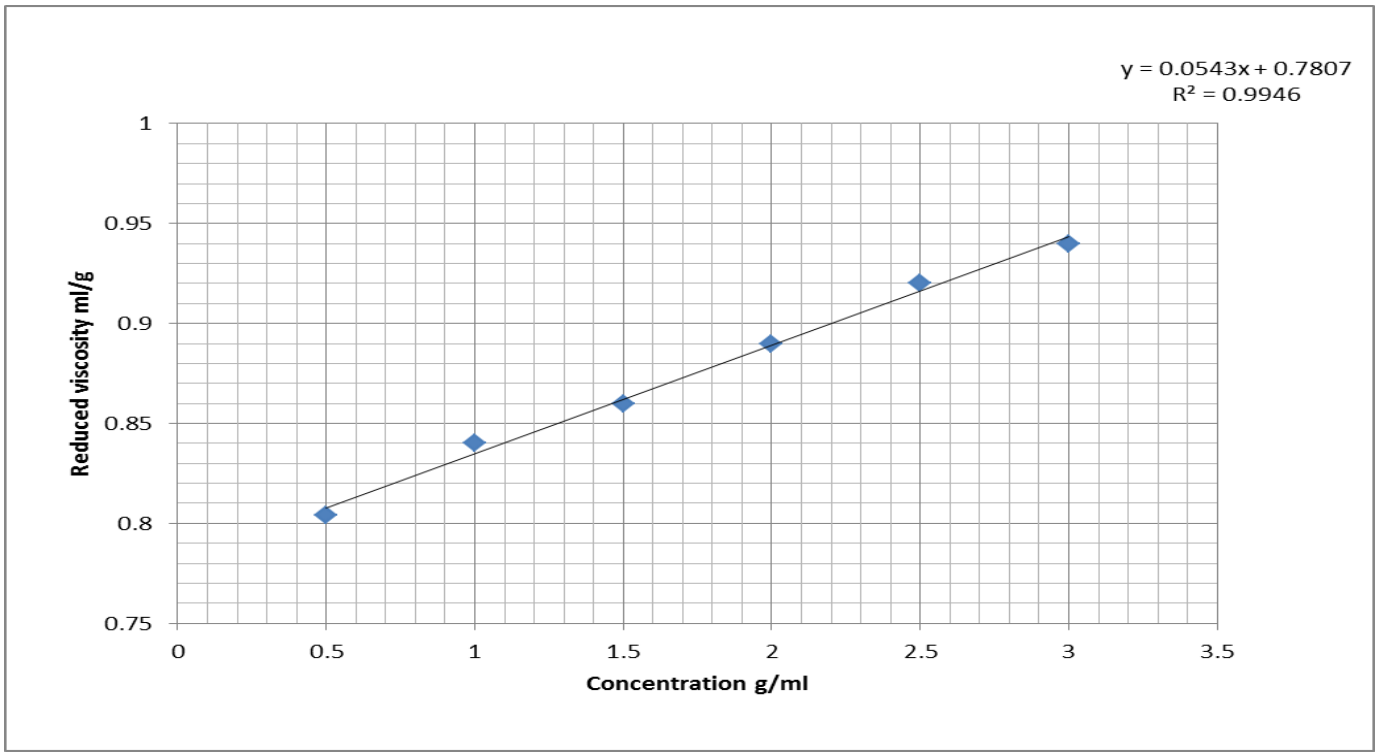
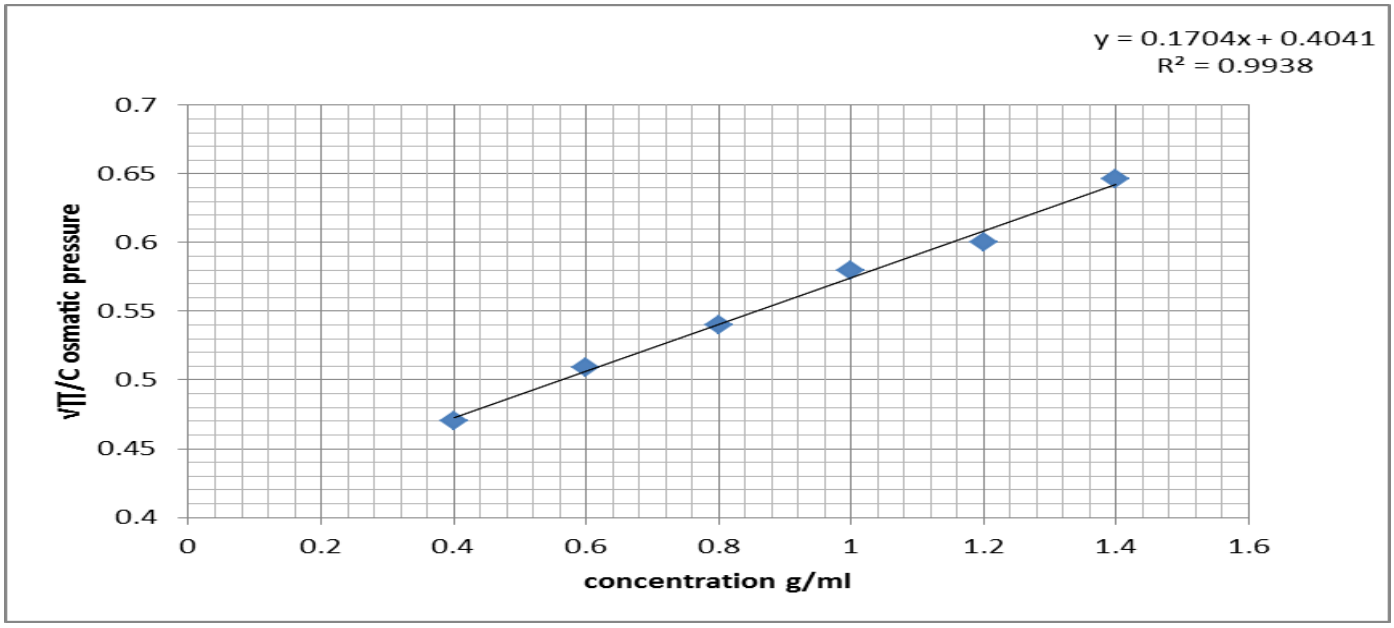
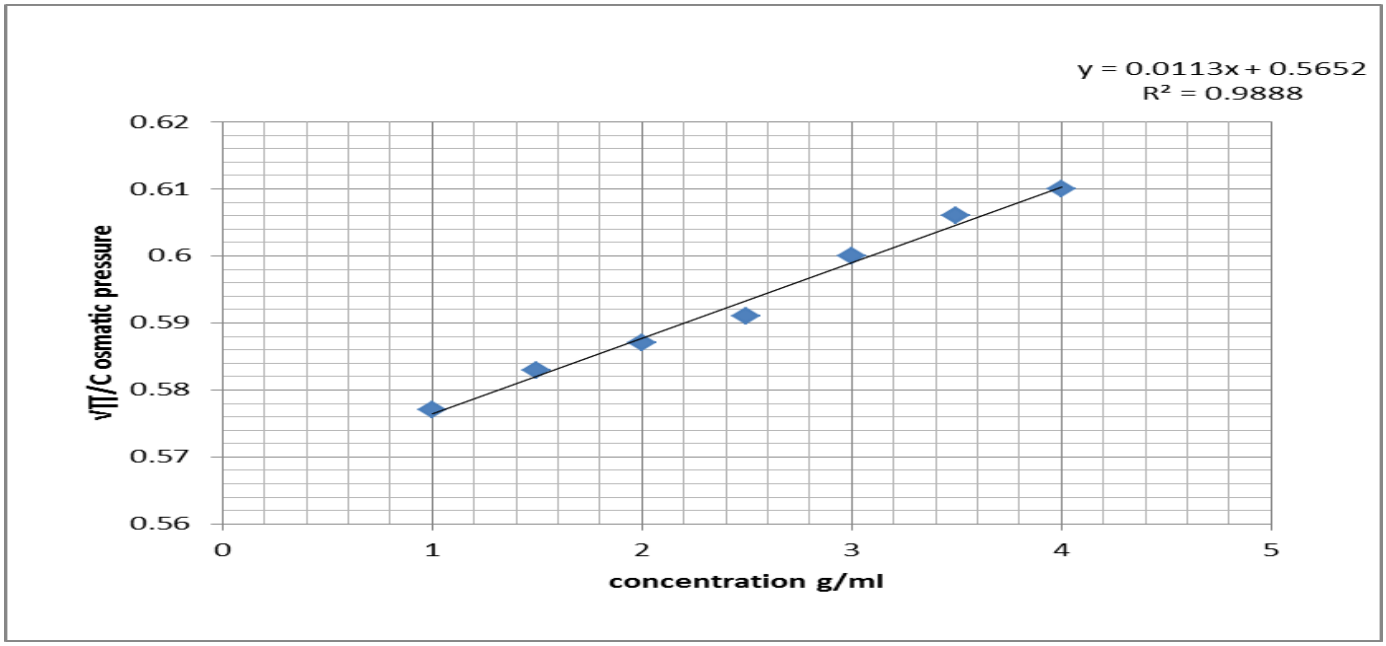


Figure (3.2) A plot of concentrations as a function of reduced viscosity for hydrophobic fraction samples



Figure(3.3) A plot of concentrations as a function of osmatic pressure for hydrophilic fraction samples



Figure(3.4) A plot of concentrations as a function of osmatic pressure for hydrophobic fraction samples

3.2 Cationic compositions

Table(3.6) shows the cationic composition of crude *Acacia polyacantha* gum and its fractions(HI and HO fractions)

Sodium content of HI fraction showed 39µg/g which is less than of the value of 43 µg/g reported by Omer(2004) for *A. polyacantha* gum. HO fraction showed 7.1µg/g which is lower than reported by Omer(2004) for *A. polyacantha* gum

Potassium content of HI fraction 130µg/g, which higher compared to HO fraction (109.65µg/g), However, Omer(2004) reported slightly lower mean value of 0.311µg/g. for A. *polyacantha* gum. Magnesium of HI fraction was found to contain 14µg/g while HO fraction 15.3µg/g, However, Omer(2004) reported slightly lower mean value of 0.941µg/g. for *A. polyacantha* gum.

While calcium of HI fraction was found to contain 100µg/g, while HO fraction 90µg/g However, Omer(2004) reported slightly lower mean value of 1.28 µg/g. For A. *polyacantha* gum

Iron content of HI fraction was found to be 2.4µg/g, while HO fraction showed 1.5µg/g, However, Omer(2004) reported slightly higher mean value of 28.1µg/g. for *A. polyacantha* gum

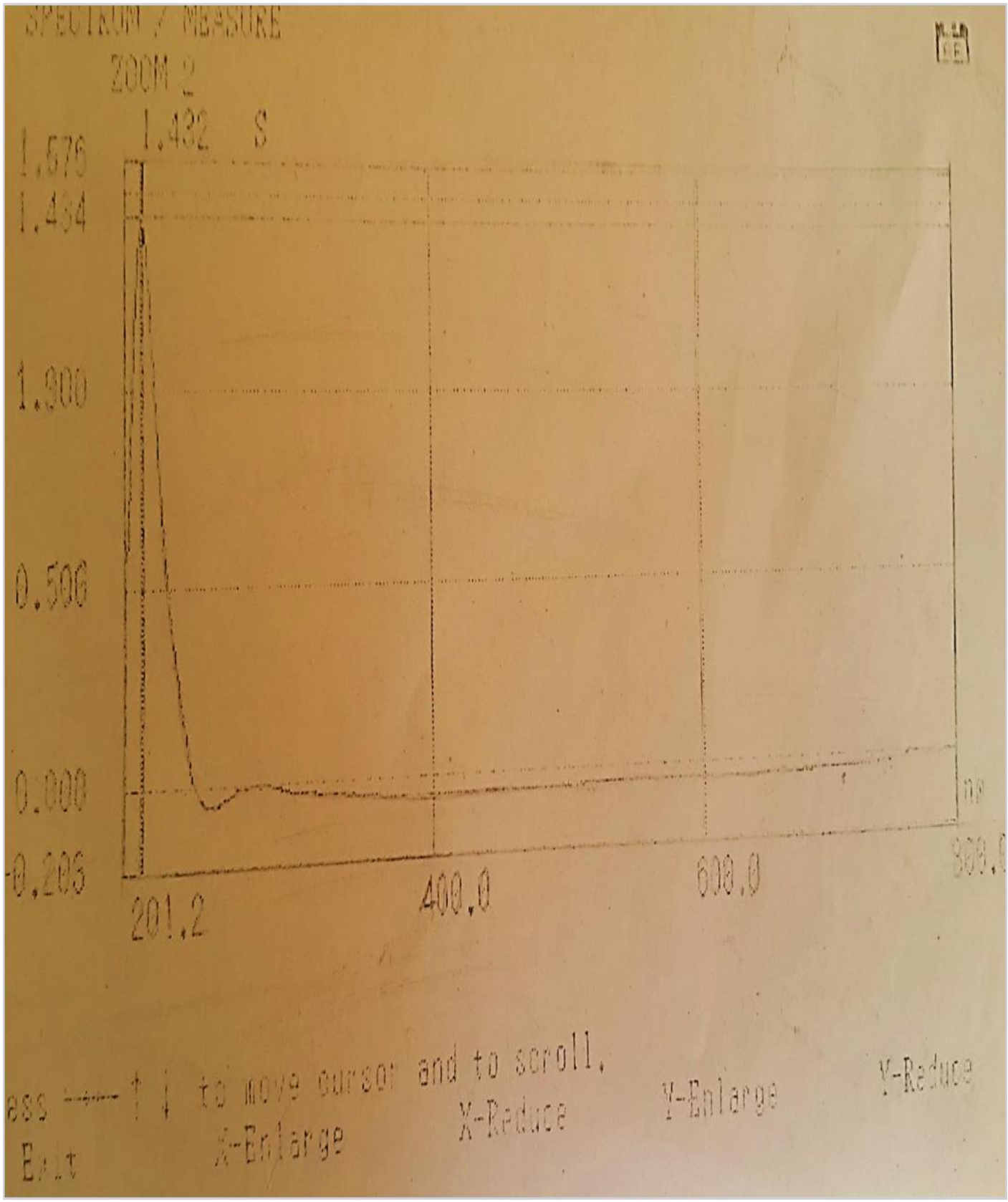
Table(3.6) Cationic composition(µg/g) of *Acacia polyacantha* gum and fractions

Metals / Samples	Na	K	Mg	Ca	Fe
Hydrophobicfraction	**7.1**	**109**	**15.3**	**90**	**1.5**
Hydrophilic fraction	**39**	**130**	**14**	**100**	**2.4**
Crude gum*	**43**	**0.311**	**0.94**	**1.28**	**28.1**

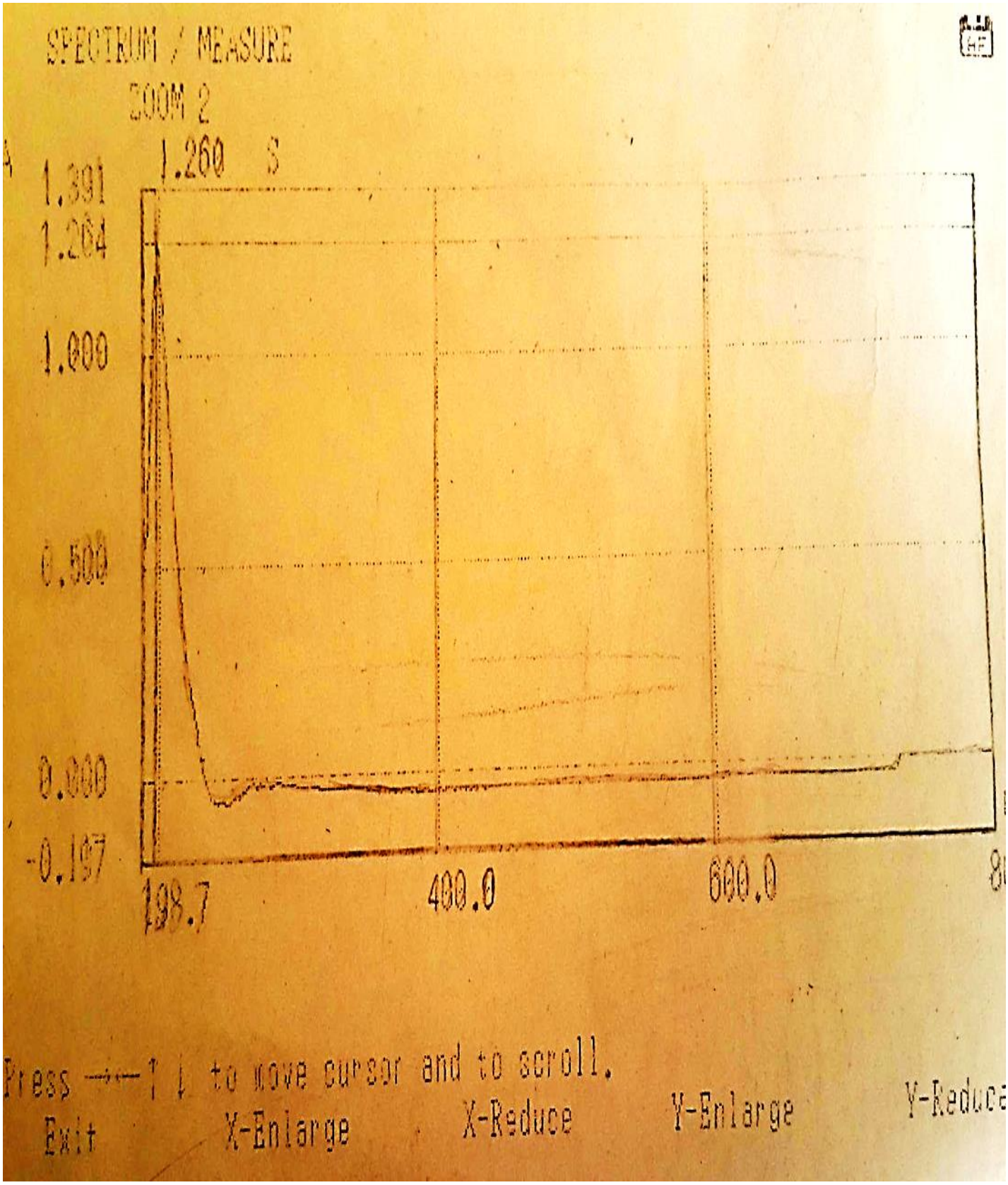
*Omer (2004)

3.3 UV Absorption

Figures 3.5 and 3.6 show UV absorption of hydrophobic and hydrophilic fractions, respectively. It was observed that maximum was approximately the same (HI =198.7nmand HI = 201.2nm), and this may prove to be a diagnostic feature and therefore an apparent analytical parameter for fractions of *A. polyacantha* gum. Omer (2004) investigated *A. polyacantha* gum reported that the absorbance is 280nm,Figure(3.7), which is higher value of absorbance than fractions of *A. polyacantha* gum.



Figure(3.5) UV spectrum for hydrophobic fraction of *Acacia polyacantha* gum



Figure(3.6) UV spectrum for hydrophilic fraction of *Acacia polyacantha* gum

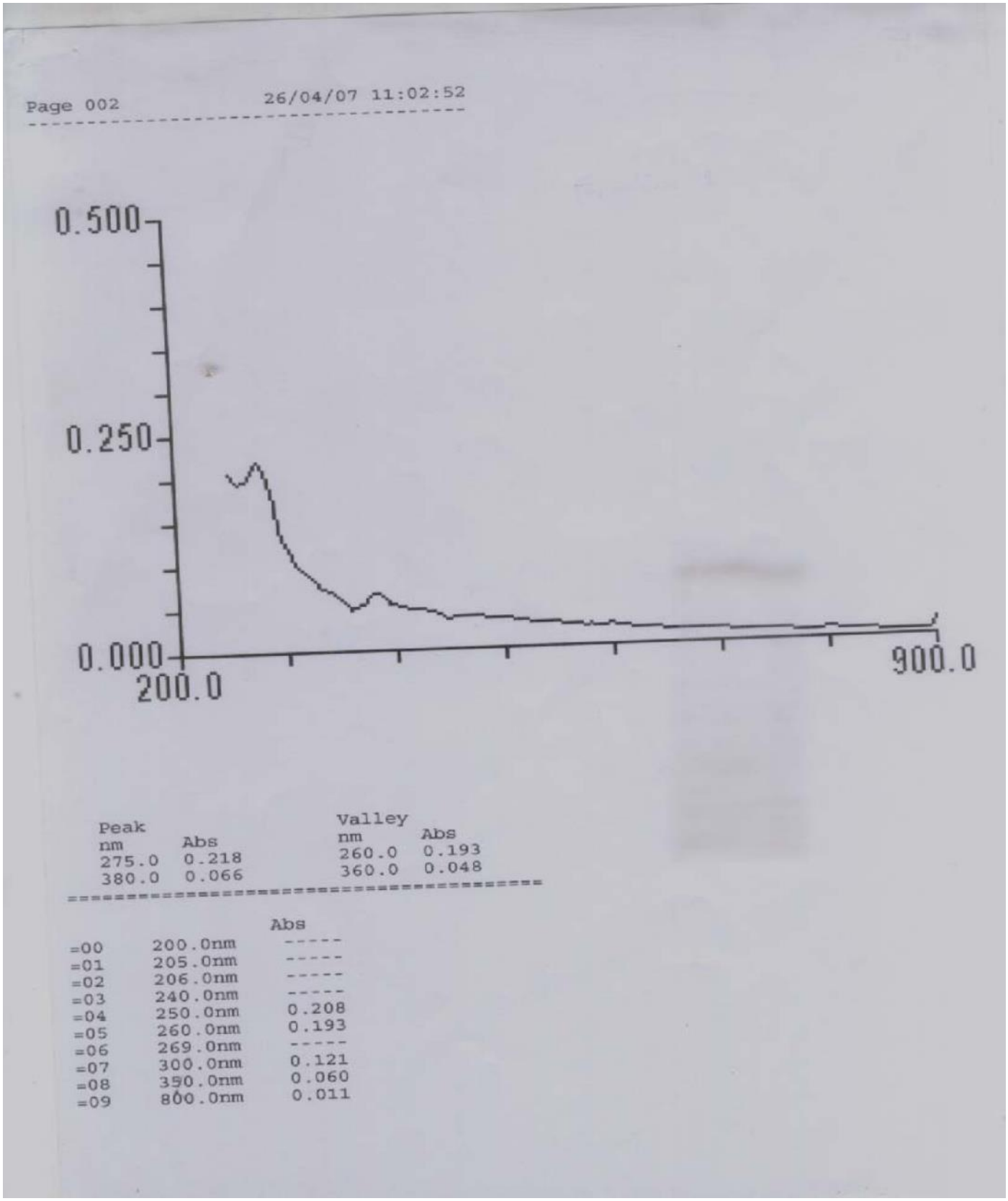
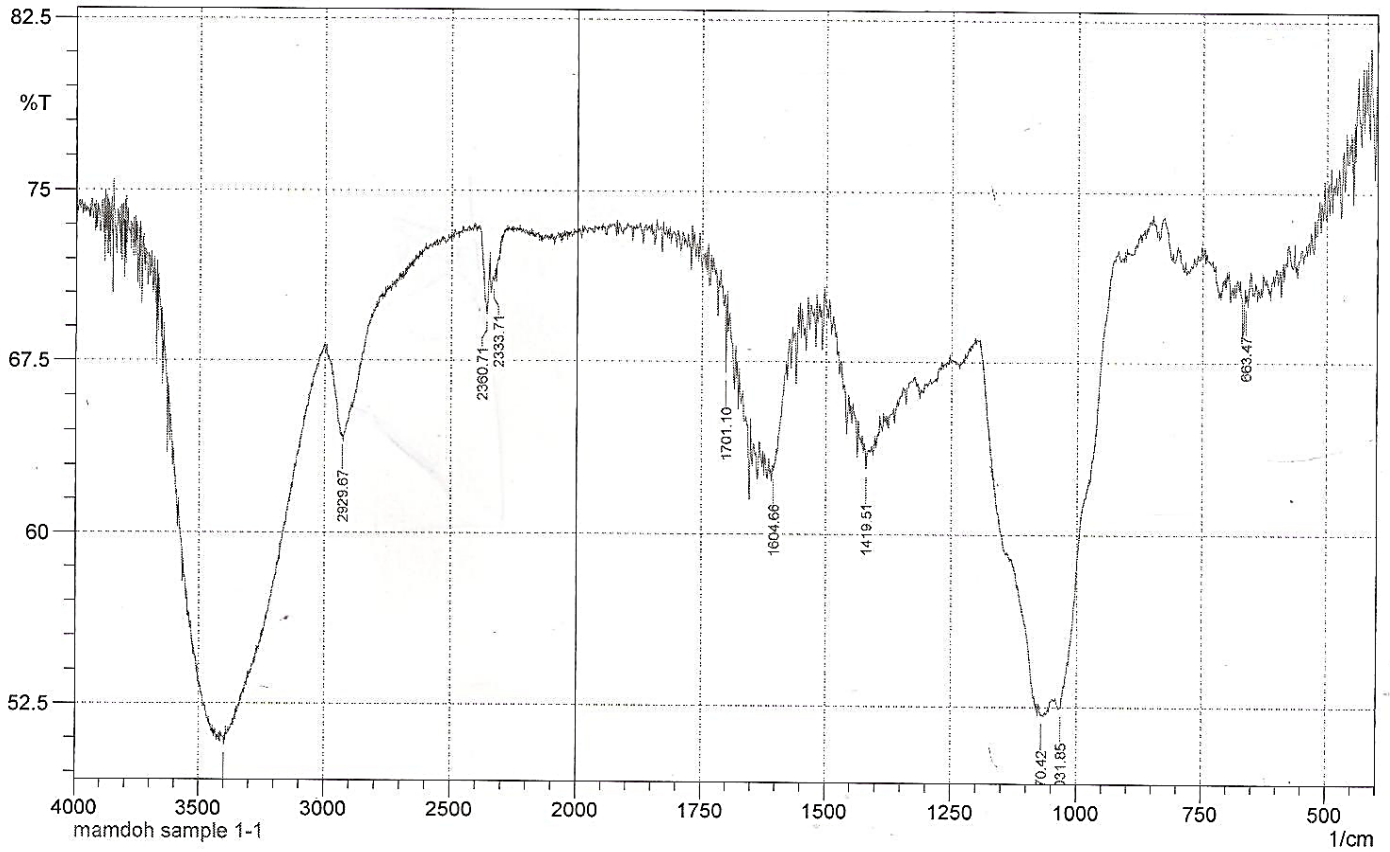


Figure (3.7) UV absorption spectra of *A. polyacantha* gum ,(Elnour.A.H, 2007).

3.4 Infra-red(IR) spectral analysis

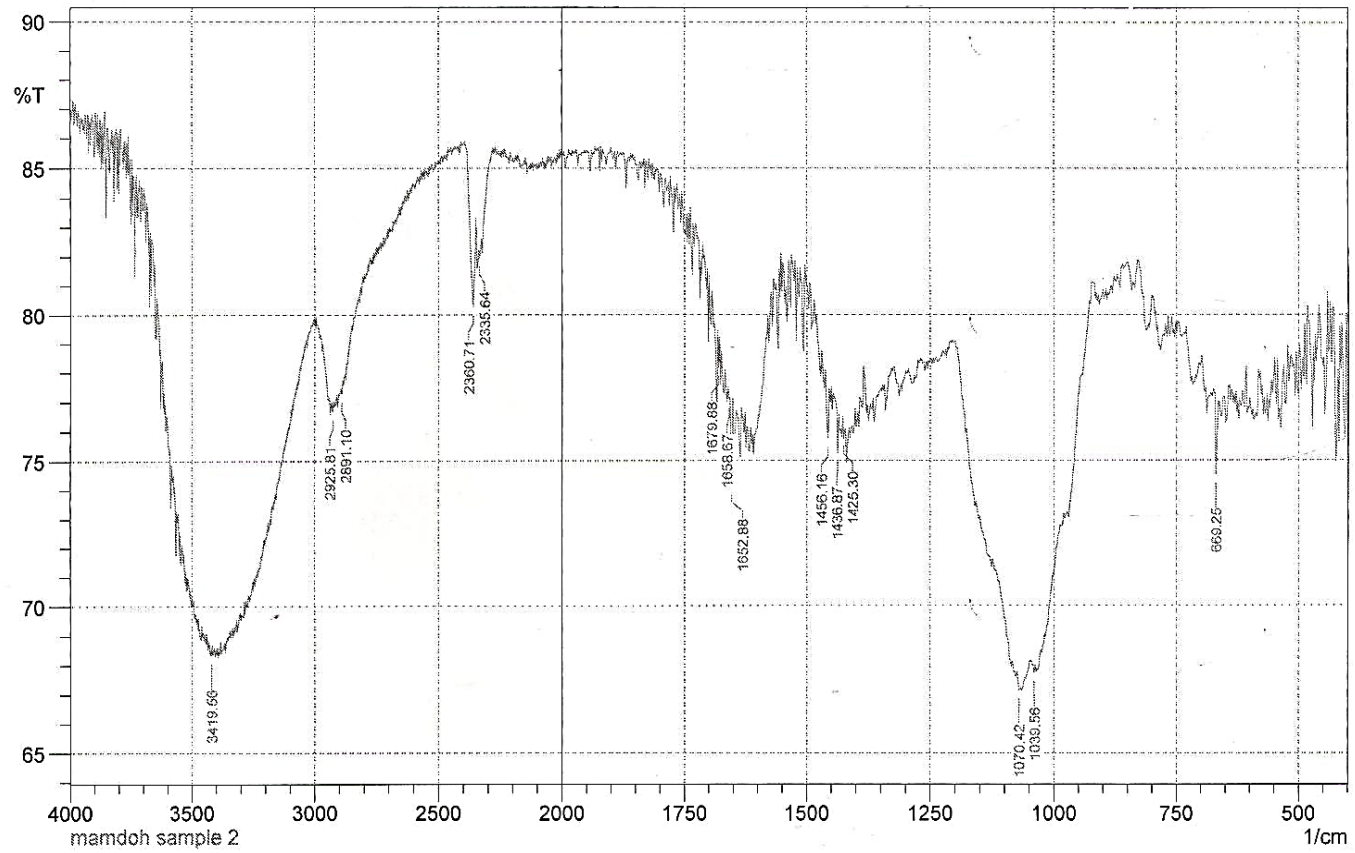
The infra-red (IR) spectra for samples of hydrophobic and hydrophilic fractions in Figures3.8 and 3.9respectively where show almost identical functional groups. The IR spectra showed six peaks with abroad one at about 3419.45cm^{-1} most likely for hydroxyl groups (OH) and two at 2925.81cm^{-1},2891.1cm^{-1} probably for aliphatic groups(C-H), also two peaks at (2333.71cm^{-1} and 2360.71cm^{-1}) likely for stretching alkynes groups and two peaks also at 1652.96$cm^{-1}$and 1658.67 cm^{-1} probably for ketone(C=O) groups, the remaining peaks at (1070.34 – 669.25 cm^{-1}) were specific for *A. polyacantha* gum. Similarly the FT.IR spectra for *A. polyacantha* gum Figure (3.10) showed peaks with broad one at about 3274.44cm^{-1}most likely for hydroxyl groups(OH), 2922.99 cm^{-1} probably for aliphatic groups(C-H)and one peak also at 1617.96cm^{-1} probably for ketone(C=O) groups.in region1420cm^{-1} are due to (-CH_2-) bending. The remaining peaks (at 1066.34 – 599.13cm^{-1}) were specific for *A. polyacantha* gum.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	663.47	70.232	0.45	665.4	661.54	0.587	0.005
2	1031.85	52.462	0.294	1033.77	1026.06	2.137	0.009
3	1070.42	52.145	0.152	1076.21	1068.49	2.171	0.006
4	1419.51	62.885	0.977	1423.37	1413.72	1.9	0.02
5	1604.66	62.834	0.356	1606.59	1598.88	1.542	0.014
6	1701.1	67.05	3.502	1703.03	1699.17	0.627	0.043
7	2333.71	71.031	0.321	2337.56	2327.92	1.424	0.013
8	2360.71	69.6	3.022	2389.64	2349.14	5.938	0.371
9	2929.67	64.264	0.059	2931.6	2927.74	0.74	0.001
10	3398.34	50.694	0.404	3402.2	3394.48	2.26	0.01

Date/Time; 12/15/2015 11:50:07 AM
No. of Scans; 10
Resolution; 4 [1/cm]
Apodization; None

Figure(3.8) FT-IR spectrum for hydrophobic fraction of *Acacia polycantha* gum



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	669.25	74.547	2.66	675.04	663.47	1.348	0.048
2	1039.56	67.739	0.243	1049.2	1037.63	1.939	0.004
3	1070.42	67.158	0.327	1074.28	1066.56	1.327	0.009
4	1425.3	75.804	0.574	1427.23	1421.44	0.688	0.009
5	1436.87	75.285	1.4	1446.51	1434.94	1.334	0.016
6	1456.16	75.734	1.851	1461.94	1454.23	0.892	0.048
7	1652.88	74.177	2.936	1656.74	1650.95	0.703	0.05
8	1658.67	77.084	0.219	1660.6	1656.74	0.434	0.002
9	1679.88	78.182	1.142	1681.81	1677.95	0.4	0.012
10	2335.64	81.979	0.356	2339.49	2325.99	1.147	0.013
11	2360.71	80.293	3.657	2395.42	2349.14	3.715	0.349
12	2891.1	77.262	0.268	2893.02	2887.24	0.643	0.005
13	2925.81	76.686	0.233	2927.74	2923.88	0.442	0.003
14	3419.56	68.364	0.24	3421.48	3417.63	0.634	0.003

Date/Time; 12/15/2015 12:00:12 PM
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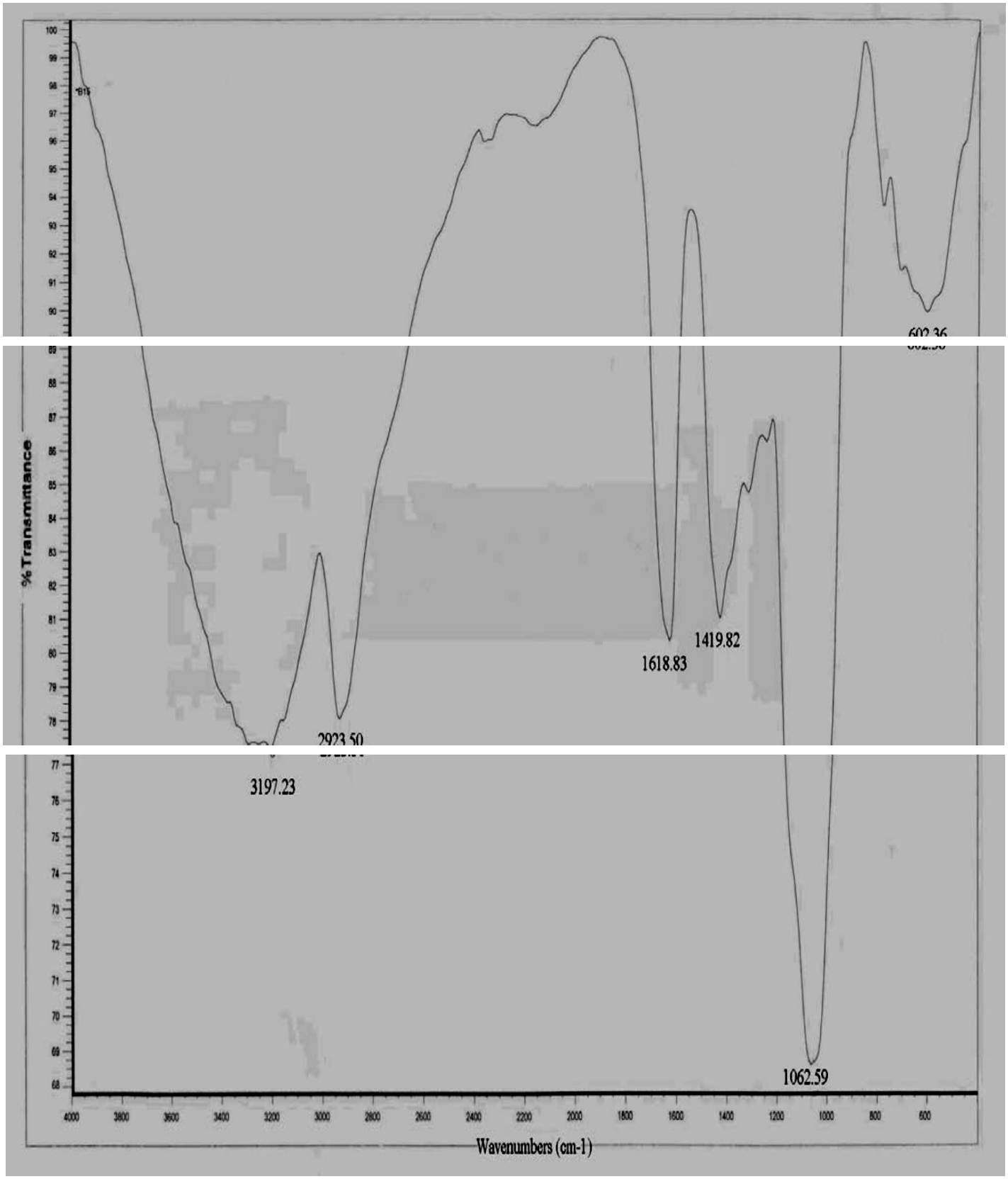
Resolution; 4 [1/cm]
Apodization; None

Figure(3.9) FT- IR spectrum for hydrophilic fraction of *A.polyacantha* gum



Figure(3.10) FT-IR spectrum for *Acacia polyacantha* gum,(Elnour. A.H,2007).

3.5 Conclusions

The physical, chemical analysis of two fractions formulations showed that:

1. Hydrophobic fraction has high calcium and potassium content, so that it can be used for nutritive values**.**

2. Hydrophobic fraction has high protein content so we can use it in wide applications Such as food and Pharmaceutical industry

3. From all the data obtained it is clear that the two fractions of *polyacantha* gum investigated differ in their composition, structure and physicochemical properties, therefore it is logical to set a seperate specification for each fraction. i.e. a seperate monogram and seperate INS numbers.

4. The high molecular weight and high solubility were indicate this fractions of *Acacia polyacantha* gum are high branched

3.6 Recommendations:

1. Further research efforts should be directed to elucidate the molecular structure of fractions of *A.polyacantha* gum.

2. Study the sugar and amino acid composition of the fractions

3 For industrial application, the fractions are recommended to be used preferably as spray-dried form

3. Further work on other functional properties for fractions of *Acacia polyacantha* gum is needed to see the possible more areas of application.

4. New production of gum fractions needs to be developed method for bulk

3.7 References

Abdelrahim,B.E.(2006). The usage of Gum Arabic Fraction and Dietary Fibers for Chronic Renal Failure Patients. Ph.D, Thesis, Faculty of Pharmacy, University of Khartoum, Sudan

Abdelsalam, Z.(1998). Gum Exudates as Taxonomy for Classification of Some Sudan *Acacias*. M.Sc. Thesis, Faculty of Sciences, University of Khartoum, Sudan

Amin,H.M.(1977). Forest Administration, *Forest Research Institute, Bulletin*, No**2**, Khartoum, Sudan.

Anderson, D.M.W., Dea, I.C.M and Karmalla, A.K. (1968),*Carbohydr,Res,*6, p 97-103

Alaa,M.M.(2015).Physiochemical and Thermodynamic Study of *Anogeissus Leiocarpus* Gum.M.Sc. thesis, Sudan University of Science and Technology

Amin, H.M. (1977), Forest Adminstration , Forest research institute Bulletin , No 2, Kharrtoum .sudan

Anderson, D.M.W. (1986). Nitrogen conversion factors of the proteinaceous content of gums permitted as food additives and contaminants. No**3**,p 231–234.

Anderson, D. M. W and Mcdougall, F. J.(1985).the proteinaceous components of the gums from some *Acacia* species. *Photochemistry*,No **24**, p 1237-1240.

Anderson,D.M.W(1994).The chemistry and Industry of Forest Products.Vol**14**,No**3,** p**67**

Baker,C.A.and Williams,R.J.P. (1956). *J.chem.Soc*.p 2352

Biswas,B., Biswas.S and Phillips. G. P.(2000). The relationship of specific optical rotation to structural composition for *Acacia* and related gums .J. *Food hydrocolloids***14**, p 601-608.

Barron ,L.D, Gargaro,A.R. and Qween,Z.O.(1991). Vibrational Raman Optical Activity *Carbohydrate. Res*. **210**, pp. 39-49.

Churms, S.C,Merrifield,E.H. &Stephen,A.M.(1983).some new aspects of the molecular structure of *Acacia senegal* gum(gum arabic). *Carbohydrate.Res*.**123**,p 267-264

Daly, G.C.,Bassler,G.W. and Morrill,T.C.(1990). Spectrometric Identification of Organic copd's, 5th ed., Chapter **3**. New York

Dermyn,M.A.(1962). Chromatography of Acidic Polysaccharide on DEAE. *Cellulose. Australian Journal of Biological Science*, Vol.**5**, p 787-791

Dickinson,E,Murray,Stains and Anderson,D.M.W.(1988),*Food Hydrocolloides*,**3**, p 65.

Defaye, J& Wang, E.(1986), Carbohydr,Res , 150, p 221-231.

Elnour,A.H.(2007).Fractionation; Physicochemical and Functional properties of *Acacia Polyacantha* gum. M.Sc. Thesis,University of Khartoum, Sudan

Elgaili. A. Omer, Abdulaziz. A. Alomari, Mohammed. E. Osman, and Ahmed A. El-Henawy.(2015).Preparative Fractionation Analysis of *Acacia polyacantha* Gum Using Acetone as a Solvent.*American Chemical Science Journal*.NO.**6(3)** ,p 164-171

Elkhatim, K.S.(2001). Factors affecting the emulsifying properties of *Acacia* gums, MSc Thesis .U. of Khartoum, Sudan

Etayeeb,S.A. (1999).Analytical studies on the gum exudates from *Anogeissus Leiocarpus*, Ph.D. Thesis U. of .Khartoum, Sudan

Gehle, R.D. and Schügerl, K.(1984). Protein Recovery by continuous flotation. *Microbiol. Biotechnol*, **20**, p 133-138

Glieksman, A.M and Saud, R.E. (1973). In whistler, R. L. ed "Industrial Gums" 2nd ed. Academic Press, New York

Heba, A.N.(2009) " Effect of Seeds source and Soil type on Seeds germination and Seedlings Establishment of *Acacia polycantha* (Willd) North Kordofan State, Sudan", M.Sc. thesis, University of Khartoum

JECFA(FAO), Rome (1990). *Specification for identity, and purity for certain Food Additives, food nutrition* Paper No, **49**; p 23-35.

Karamalla,K. A.(1965).Analytical and Structural Studies in the polysaccharide group,

Ph .D. Thesis U of Edinburgh, UK

Karamalla,A.K.,Siddig, N.E.and Osman,M.E.(1998).Analytical data for *A.senegal var. senegal* Gum Samples Collected Between 1993 and 1995 from Sudan. *Food Hydrocolloids*, p 1-6.

Karamalla,K.A., Siddig,N.E. and Osman, M.E.(1998). *Food Hydrocolloids*,**12**,p373- 378.

Lewis, B.A.,Smith,F.(1957). *Journal of the American Society*. **79**, p 3929

Mantell.C.L.(1965).The water soluble gums.Reinhold NewYourk.

Narsimhan, G.(2000). Foam Fractionation. Purdue University, West Lafayette, IN, USA.

Osman,M.E,Menzies,A.R,Willimas,.A.R&philips,G.O.(1994).”*Food Hydrocolloids*”,**8,**p 225-242

Omer, E.A.(2004). Characterization and Analytical Studies of *A.polyacontha* Gum, Ph.D. Thesis, Sudan, University of Science and Technology, Khartoum, Sudan.

Omer ,A.A.A.(2015), The Thermodynamic Properties of Some *Acacia* Gums. Ph.D. Thesis, Sudan university of Science and Technology, Khartoum, Sudan

Paul Stevenson and Xueliang Li,(2014),” Foam Fractionation Principles and Process Design”, Taylor & Francis Group, P 28-29

Siddig, N. E.(2003). Characterization, Fractionation, Functional Studies on Some *Acacia* Gums; Ph. D. Thesis,University of Khartoum

Siddig,N.E.(1996). Nitrogen and specific rotation as qualifying indices for Gum Arabic drived from *Acacia Senegal* (L.)willd, M.Sc.Thesis. U.of Khartoum, Sudan

Sharma,S.C.(1981).Gums and Hydrocolloids in oil water Emulsions. *Food Technology. p*59- 67

Smith, F. And Montgomery, R.(1959). The chemistry of plant gums and mucilages. *Reinhold*. New York

Stephen, A.M.; Nunn, J.R. and Charles, A.J.(1955). *J. Chem. Soc*., p 1428

Street, C. A.; and Anderson, D. M. W.(1983) Talanta,**30,** p 887-893

Stoddart, J.F.; Andersn, D.M.W.(1966b). Studies on Uronic Acid Materials: Part XV. The Use of Molecular Sieve Chromatography in Studies on *Acacia senegal* Gum. *Carbohydr. Res*. **2**: pp. 104-111

Stanely, H.P& James,B.H.(1980).Hammond-organic Chemistry, 4th ed. N.Y. p 130-195.

Voget, K.(1995). Common Trees and Shrubs of Dryland. Sudan, London

Williams,P.A, Phillips, G.O. and Randal, R.C.(1989). *Food Hydrocolloids*, **3**: p 65-75.

3.8 Appendixes

Table(3.2) Reduced viscosity of different concentration of hydrophilic fraction solution

C g/ml	Tsec	T/T_0	$(T/T_0)-1$	$((T/T_0)-1)/C$
0.05	69.18	1.026	0.026	0.53
0.1	71.84	1.066	0.066	0.66
0.15	75.08	1.113	0.113	0.76
0.2	79.26	1.176	0.176	0.88
0.25	84.08	1.247	0.247	0.99

Table(3.3) Reduced viscosity of different concentration of hydrophobic fraction solution

C g/ml	T_{sec}	$(T/T_0)-1$	$((T/T_0)-1)/C$
0.5	91.13	0.402	0.804
1	120	0.846	0.84
1.5	149	1.26	0.86
2	180.7	1.78	0.89
2.5	215	2.3	0.92
3	248	2.82	0.94

Table(3.4) Osmotic pressure of different concentration of Hydrophilic fraction solution

Concentration g/ml	Osmatic pressure ∏(mm Hg)	∏/C	√∏/C
0.4	0.08	0.22	0.47
0.6	0.156	0.26	0.509
0.8	0.25	0.31	0.54
1.0	0.34	0.34	0.58
1.2	0.44	0.366	0.6
1.4	0.57	0.41	0.646

Table(3.5) Osmotic pressure of different concentration of hydrophobic fraction solution

C g/ml	∏	∏/C	√∏/C
1	0.33	0.33	0.577
1.5	0.51	0.34	0.583
2	0.69	0.345	0.587
2.5	0.875	0.35	0.591
3	1.08	0.36	0.6
3.5	1.288	0.368	0.606
4	1.48	0.37	0.61