Chapter One

General Introduction and Literature Review

1.1 Introduction

Acacia species known, commonly, as *Acacia*, thorn tree, whistling thorn, or wattle, is a genus of shrubs and trees belonging to the subfamily *Mimosoideae* of the family *Fabaceae*, described by the Swedish botanist Carl Linnaeus in 1773. All species are pod-bearing, with sap and leaves often bearing large amounts of tannins and condensed tannins that, historically, found and used as pharmaceuticals and preservatives. The name given by early Greek botanist-physician Pedanius Dioscorides (middle to late first century). This name derives from the Greek word for its characteristic thorns, (akis; "thorn").

The genus *Acacia* contains roughly 1300 species, about 960 of them are native to Australia, with the remainder spread around the tropical to warm-temperate regions of both hemispheres, including Africa, southern Asia, and the Americas (Gideon and Figueiredo, 2011). However, in 2005, the genus was divided into five separate genera under the tribe "*Acacieae*". The genus Acacia was retained for the majority of the Australian species and a few in tropical Asia, Madagascar, and Pacific Islands. Most of the species outside Australia, and a small number of Australian species, were reclassified into Vachellia and Senegalia. The two final genera, Acaciella and Mariosousa, each contains about a dozen species from the Americas.

1.2 Definition of gum

Gums are hydrophilic polysaccharides of high molecular weight, usually, with colloidal properties, which in an appropriate solvent may dissolve or swell to produce gels or, highly, viscous suspension, thus the term gum is applied to a wide variety of substances of "gummy" characteristics and cannot be precisely defined. Hydrophobic substances often called gums are high molecular weight hydrocarbons and other petroleum products, rubbers, certain synthetic polymers, chicly for chewing gum, and the resinous saps which often exude from evergreens and which are sometimes commercially tapped yielding, for example, gum balsam and gum resin (Anderson *et. al.*, 1968).

Most commonly, the term gum as technically employed in industry refers to plant polysaccharides or their derivatives which are dispersible in either cold or hot water to produce viscous mixture or solutions. Thus, modern usage includes the water – soluble or water – swellable derivatives of cellulose and the derivatives and modifications of other polysaccharides which in the natural form are insoluble. Usage would classify as gums all polysaccharides or their derivatives which when dispersed in water at low dry substance content, swell to produce gels, highly viscous dispersions, or solutions (Coppen, 1995). This definition does not require that gums have the property of tackiness, and consequently, such a definition includes as gums those polysaccharides and derivatives which are slimy or mucilaginous. Some authors have tried to classify, separately, these slimy substances from plants into a category called mucilage. Yet, it is more logical to consider tackiness and sliminess as the exhibition of two different physical properties of gums. Hence, there are tacky gums and slimy or mucilaginous gums. Tackiness and sliminess are manifestations of two, somewhat, controllable physical properties. It is possible

to modify a gum so that tacky properties are withdrawn and mucilaginous properties introduced. Yet the gum remains hydrophilic and capable of giving high viscosity to its dispersions even at low concentrations, consequently, the mucilaginous property is distinctive but a category of mucilage's has no chemical significance (Glicksman, M. 1969).

It has been customary in the past to classify most gums as polysaccharides and to group them according to plant origin. Thus, the seaweed group comprised the extracts known as agar, alginates, and carrageenan; tree exudates are gum Arabic, gum karaya, gum tragacanth, and gum ghatti; seed gums include locust bean and guar gum. The other gumlike materials such as pectin and starch were treated as separate groups, while gelatin, being a protein was not included at all. In addition, there was no room for synthetic gums such as cellulose derivatives which are carbohydrate gum, or for the synthetic vinyl polymers such as polyvinylpyroldinone (PVP) which require a completely new category. The use of botanical origin as a basis for the classification of important plant gums is valid and useful, since gums of similar origin and functionality, frequently, have similar properties and chemical structures, and can, occasionally, be employed for the same purpose. Thus, locust bean gum and guar gum, which are both derived from similar plant- seed sources, have similar chemical structure of neutral Galactomannans, and differ only in the ratio of Galactose to mannose molecules. Their thickening properties are, sufficiently similar and of the same magnitude to allow the interchange of the gums in certain specific applications, but not indiscriminately in all applications. For a general classification to be useful, it should embrace all types of gums that are used in the food industry, and it should leave room for new gums that are certain to be developed in the future. Following this line of thought it has been proposed that

the following all–inclusive classification composed of three main categories. (1) natural gums – those found in nature; (2) modified natural , or semi synthetic gums – those based on chemical modifications of natural gums or gumlike materials ; (3) synthetic gums – those prepared by total chemical synthesis (Table 1.1). As a further aid to identification each category is broken down into subgroups based, where possible, on the common origins, functions, or properties of the particular gums (Whistler, R. L., 1973).

Table 1.1 Classification of gums

Natural gums	Modified (semisynthetic) gums	Synthetic gums
Plant exudates: Arabic Tragacanth Karaya Ghatti	Cellulose derivatives: Carboxymethcy cellulose Methylcellulose Hydroxypropylmethyl- Cellulose Methylethylcelulose Hydroxypropylcellulose (klucel)	Vinylpolymers: Polyvinylpyrrolidone (PVP) Polyvinyl alcohol (PVA) carboxyvinyl polymer (carbopol) Ethylene oxide polymers: Polyox
Plant extracts: Pectins Arabinogalactan (larch gum)	Low methoxy pectin	
Plant seed flours : Locust Bean	Microbial fermentation gums: Dextran Xanthan gum	

Quar		
Psyllium seed		
Quince seed		
<u>Seaweed extracts</u>:		
Agar		
Alginates		
Carrageenan		
Furcellaran		
	Pregelatinized starches	
<u>Cereal starches</u>:		
Seed starches		
Corn		
Wheat	Pregel at inlzed starches	
Rice	Modified starches:	
	Carboxymethyl starch	
Waxy maize	Hydroxethyl starch	
Sorghum	Hydroxypropyl starch	
Waxy sorghum		
Tuber starches		
Potato		
Arrowroot		

1.3 Classification of the *Acacia* genus

There are over 1,300 species of *Acacia* distributed around the world (Fig. 1.1), the traditional circumscription is not monophyletic. This discovery has led to the breaking up of *Acacia* into five genera, along with the much-debated retypification of the genus with an Australian species instead of the original African type species, an exception to traditional rules of priority that required ratification by the International Botanical Congress (Gideon F. Smith, 2011). That decision has been controversial, and debate continues, with some taxonomists (and many other biologists) deciding to continue to use the traditional *Acacia* circumscription of the genus, at least for now. Such use is contrary to decisions by two consecutive International Botanical Congresses. When the genus is divided, the traditional *Acacias* of *Africa* are now treated in the genera *Vachellia* and *Senegalia*, some of the American species are placed in *Acaciella* and *Mariosousa*, and the majority of species still treated in the genus *Acacia* are confined to Australia. (Australian National Herbarium. Retrieved October 24, 2013).

Fig. 1.1   Acacia distribution around the world

In common parlance, the term "*Acacia*" is occasionally applied to species of the genus *Robinia*, which also belongs in the pea family. *Robinia pseudoacacia*, an American species locally known as black locust, is sometimes called "false *Acacia*" in cultivation in the United Kingdom and throughout Europe (Thanukos, 2009).

1.4 Botanical classification of the genus Acacia

Kingdom*:* *Plantae*

Clade*:* *Angiosperms*

Clade*:* *Eudicots*

Clade*:* *Rosids*

Order*:* *Fabales*

Family*:* *Fabaceae*

Subfamily*: Mimosoideae*

Genus*:* *Acacia*

1.5 General description of *Acacia* trees

The leaves of *Acacias* are compound pinnate in general. In some species, however, more especially in the Australian and Pacific islands species, the leaflets are suppressed, and the leaf-stalks (petioles) become vertically flattened in order to serve the purpose of leaves. These are known as "phyllodes". The vertical orientation of the phyllodes protects them from intense sunlight since with their edges towards the sky and earth they do not intercept light as fully as horizontally placed leaves. A few species (such as Acacia glaucoptera) lack leaves or phyllodes altogether but instead possess cladodes, modified leaf-like photosynthetic stems functioning as leaves.

The small flowers have five very small petals, almost hidden by the long stamens, and are arranged in dense, globular or cylindrical clusters; they are yellow or cream-colored in most species, whitish in some, or even purple (*Acacia purpureopetala*) or red (Ac*acia lepr*osa (Scarlet Blaze)). *Acacia* flowers can be distinguished from those of a large related genus, *Albizia*, by their stamens, which are not joined at the base. Also, unlike individual Mimosa flowers, those of *Acacia* have more than ten stamens. (Singh, Gurcharan, 2004).

The plants often bear spines, especially those species growing in arid regions. These sometimes represent branches that have become short, hard, and pungent, though they sometimes represent leaf-stipules. *Acacia armata* is the kangaroo-thorn of Australia, and *Vachellia erioloba* (syn. *Acacia eriolobata*) is the camelthorn of Africa.

Acacia seeds can be difficult to germinate. Research has found that immersing the seeds in various temperatures (usually around 80°C (176°F)) and manual seed coat chipping can improve growth to around 80% (Clemens, PG Jones, NH Gilbert, 1977).

1.6 Gum production

Acacia Senegal trees are tapped when they are 6 – 7 years old. Each season the bark is torn from the tree in a different place using a sharp tool. Gum is collected by hand after 6 week from tapping and every week thereafter during the dry season. Annual yields from young trees have been reported to range from 200 g to 3 Kg, with an average of 900 g; older trees give about 400 g to 7 Kg, with an average of 2000 g (Glickman, 1973). Gum Arabic exudates as a white to yellow spherical tears of varying size. It is tasteless and dissolves

readily in water, and aqueous solutions up to 50% by weight can be prepared. It is insoluble in most organic solvents (Anderson *et. al.,* 1990).

1.7 Factors affecting gum exudation.

(Blunt, 1926) reported that the largest quantities of gum are produced by unhealthy trees and this suggests that this phenomenon may be due to adaption of the tree to stress from external environment e.g. poor soil, wind, grazing animal and insects. The sahelian tree locust (*Anacridium melanorhodon*) has been reported to defoliate *Acacia* trees. If the tree is defoliated sap necessary from gum formation will not be left and in the following year the tree will be more susceptible to infection therefore gum production will be increased. Tapping induces gum formation. Good rainy season and good climatic conditions improve the vitality of the tree, which results in high gum exudation.

1.8 The Sudan gum belt

"The gum Arabic belt", where *A. Senegal* grows naturally, coincides with the area of central Sudan mainly between latitudes 10º and 14º N. The two most conspicuous gum Arabic belt areas outside these limits are the northeast (Faw - Gedaref - Kassala), and in the southeast along the Blue Nile/Upper Nile states border Fig. (1.2). The total gum belt area in *Sudan* amounts to 520,000 km², which is equal to one-fifth of the area of the country. A field survey conducted in 1989 indicated that there existed scarcely any *A. Senegal* north of latitude 13º 45´ in *Kordofan* or Darfur (HID & IES, 1990; John, 2002). The gum belt provides a buffer against desertification across the vast region of the Sudano-Sahelian zone. *A. Senegal* provides a variety of valuable economic and ecological functions, such as gum Arabic, fodder for livestock, fuel wood and shade, as well as many indirect benefits associated with the tree. Its extensive

and massive root system reduces soil corrosion, and as a leguminous tree, it fixes nitrogen which encourages grass and crop growth. The tree is also essential in sand dune fixation for these reasons; it is the preferred species in bush-fallow rotational and intercropping farming systems in the dry lands of western Sudan (Bourbon, 2010)
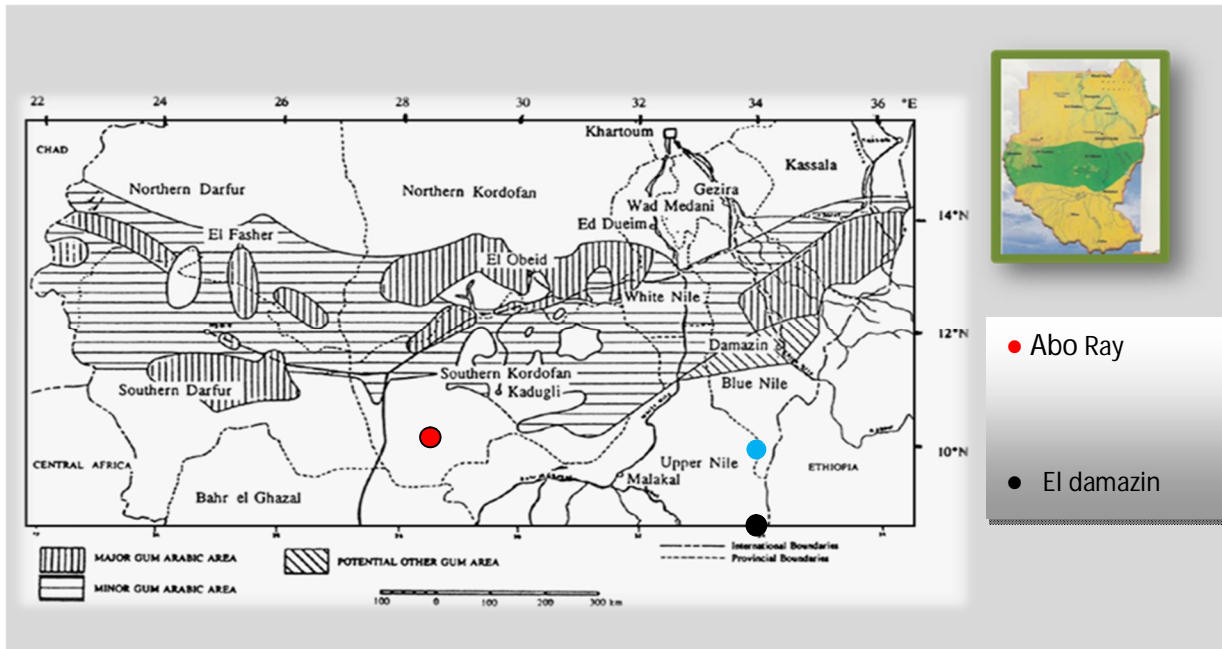

Fig. 1.2   Gum Sudan belt

1.9 *Sudan* gum producing trees

Sudan is endowed with more than 30 *Acacia* species (Table 1.2), most of which yield gum (EL-amin, 1990), species with greatest distribution that includes, *Acacia senega*l (Hashab), *Acacia seyal* (Talha), *Acacia polyacantha* (Kakamut), *Acacia laeta* (Shubahi), *Acacia mellifere* (Kitir), *Acacia nilotica* (sunt), *Acacia sieberiana* (kuk), and *Acacia oerfota* (Lao't) (Abdel Nour, 1999).

Table 1.2 Sudan gum producing trees

1	*Acacia Senegal var Senegal*	24	*Adansonia*
2	*Acacia Seyal var Seyal*	25	*Afzzelia Africana*
3	*Acacia Seyal var fistula*	26	*Albizzla amara*
4	*Acacia drepanolobium*	27	*Anogeissus schimperi*
5	*Acacia soirocarpa*	28	*Balanites aegyptiaca*
6	*Acacia Raddiana*	29	*Bauhenia reticulate*
7	*Acacia Stenocarpa*	30	*Bauhenia fassoglensis*
8	*Acacia Campylacantha*	31	*Boswellia papyrifera*
9	*Acacia Sieberiana*	32	*Cassia arereh*
10	*Acacia Arabica*	33	*Ceiba pentandra*
11	***Acacia oerfota***	34	*Cordyla africana*
12	*Acacia usambarensis*	35	*Crataera adansonii*
13	*Acacia mellifera*	36	*Danniela oliveri*
14	*Acacia albida*	37	*Isoberlinia doca*
15	*Acacia ethaica*	38	*Khaya grandifoliola*
16	*Acacia ehrenbergiana*	39	*Khaya senegalensis*
17	*Acacia ehyssinica*	40	*Lophira eleta*
18	*Acacia famesiana*	41	*Moringa pterogosperma*
19	*Acacia hecatophlla*	42	*Sterculia cinerea*
20	*Acacia lacta*	43	*Sterculia tomentosa*
21	*Poinciana elata*	44	*Tomarindus indica*
22	*Combretum splendens*	45	*Terminalia spe*
23	*Combretum collinum*	46	*Terminalia sehimperiana*

1.10 Gum collection in Sudan.

Gum *Hashab* (Fig 1.3) is collected from *Acacia Senegal* by tapping, whereas all gum *talha* from *Acacia Seyal* is collected as a result of natural and tap exudation. Tapping begins when the trees are just starting to shed their leaves, around mid of October or the beginning of November. In order to reach this stage, trees have to grow for a period of 3 to 5 years depending on the method of establishment. However, there are two tapping seasons, an earlier one before the onset of the colder weather and a later one in the dry spell after March.

After tapping, exudation occurs, gradually, forming a hard but slightly elastic nodule (N.A.S, 1980; Anonymous, 1980-2008).

1.11 Some types of gum

Gum Arabic is defined as the production of gum from *acacia senegal*. This is the best in quality and highest in price. This is the first definition of gum arabic set in 1978 by the JECFA (Joint Committee of Expert for Food Additives) as consultancy group of the FAO and the WHO. Aid gum organization managed to convince JECFA to modify the specification and include the gum of *acacia seyal*, locally known as Talha. Sudanese Gum Arabic Company rejected that specification of each type of gum was separated. In 1999, the Codex Alimentarius Commission held its annual meeting in the Hague in Netherlands and anew text defining gum arabic declared as that of *acacia senegal* as equally as acacia seyal. The *Sudanese* scientific don't agree with this definition and argued to changed it when optical rotation and nitrogen content were introduced to the specification in 1999 (Osman, M. E. 2005).
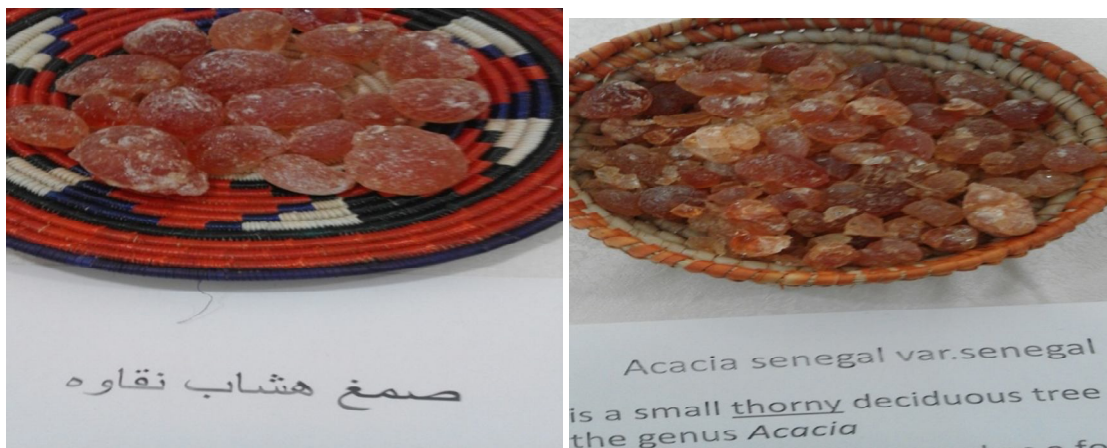

Fig. 1.3 A. *senegal* **sample**

Gum ghatti (Indian gum) is exudates from Anogeissus latifolia, a tree that is found in India and Sri Lanka. The exudations are natural, but the yield can be increased by making artificial incisions. Gum chatty occurs naturally as calcium and magnesium salt of a complex polysaccharide acid complex. Acid hydrolysis has shown the gum to consist of L-arabinose, D-galactose, D-mannose, D-xylose and D-glucuronic acid. On dispersion in water, gum ghatti forms viscous solution of viscosity intermediate between gum Arabic and gum karaya. Gum ghatti have emulsification and adhesive properties equivalent to or superior to those described for gum Arabic (Meer *et. al.,* 1973).

Gum tragacanth is the dried exudates of several species of the genus Astaragulus (family Leguminoosae). Astaragulus species is a small perennial shrub with a relatively large tap root, which, along with the branches, is tapped for gum. Gum tragacanth does not dissolve in water, but absorbs a large amount of water and swells greatly to form a soft adhesive gel. The soluble fraction of the gum is a complex mixture of acidic polysaccharides that consists of D-galacturonic acid. On hydrolysis the gum produces D-galactose, 6-deoxy-galactose, and D-xylose. Gum tragacanth powder is white to pale yellow and odorless (Coppen, 1995).

Gum Karaya (Fig. 1.4) is the name given to the dried exudation of the Sterculia urens tree. Almost a11 Gum karaya comes from India. In the early days of its importation into the United States, many types of gums were introduced which had properties similar to those of Gum karaya (Eljack,. 1999) in its monographs of "Drugs, Chemicals and Preparations," "The National Formulary" named the gum Sterculia gum, with the alternate name of Gum karaya. It described Sterculia gum as the dried, gummy exudation from Sterculia urens (Roxburgh), Sterculia villosa (Roxhurgh), Sterculia tragacantha (Lindley) or other species of

Sterculia (Sterculiaceae family) or from Cochlospermum gossypinm De Candolle or other species of Cochlospermum (Bixaceal family).' Howes established the fact that the gum shipped from India as Gum karaya is obtained from the Sterculia urens tree.' other gums of the Sterculia type are not collected commercially (Meer, W. 1980) .The gum of cochlospermum gossypium is very similar to Karaya Gum and is marketed in India (Alamin, 1999).

Fig. 1.4 Gum karaya sample

Acacia nilotica **gum** has naturally exudates by *acacia nilotica* species. *Acacia nilotica* trees extend from South Africa to Egypt and from Arabia to Pakistan and India. Introduced widely throughout the tropics and becoming neutralized, such as in the West Indies. Large areas of forests established in India and Pakistan. Planted in Africa along the Blue Nile in the Sudan, in the bushveld of Natal and Transvaal, in Zambia and Botswana. *Acacia nilotica* gum gave a high, positive specific rotation ($+106^0$), a high methoxyl content (1.05%), and contained only traces of rhamnose (Anderson *et al.*, 1966). *Acacia nilotica* gum gave solutions of low viscosity, and it's unusually low nitrogen content (0.08 %). (Anderson *et al.*, 1963, 1966).

Acacia polyacantha **gum** was studded by Anderson and Munro (1970) carried out five successive Smith-degradations; the polysaccharides obtained after each degradation were examined by methylation and linkage analysis, the polysaccharides containing D-galactose (54%), L-arabinose (29%), L-ramnose (8%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%), Linkage analysis gives 3-*O*-α-D-glactopyranosyl-L-arabinose, 3-*O*-β-L-arabinopyranosyl-L-arabinose, 3-*O*- β-L-arabinofuranosyl-L-arabinose, 3-*O*- β-D-galactopyranosyl-D-galactose, and 6-0- β-D-galactopyranosyl-D-galactose. The 0-methyl derivative of the gum was examined, after methanolysis, by G.L.C.; 2,3,4-tri-methyl-L-rhamnose, 2,3,5- and 2,3,4-tri-, and 2,5-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, and 2,4-di-*O*-methyl-D-galactose, and 2,3,4-tri- and 2,3-di-*O*-methyl-D-glucuronic acid were detected.

1.12 Chemical composition of *Acacia* gums

The work done, so far, on different types of gum from *Acacia* origin e.g. *Acacia Senegal* (Osman. 1993), *Acacia Seyal* (Hassan. 2000) and *Acacia polyacantha* (Omer. 2004) suggest that Acacia gums are complex mixtures of some metallic salts of polysacchardic acid and protein. They consist of five main sugars: galactose, arabinose, rhamnose, glucuronic acid, and 4-O-methylglcuronic acid.

The main minerals elements found are: Calcium, Potassium, Magnesium, and Sodium. Other minerals elements such as Manganese, Iron, Aluminum, Zinc, and Chromium may also present but with very small amount. The percentage of each constituent in each type of *Acacia* gum is different. Hence the structures are different, and consequently the functionality and molecular characteristic are different (Table 1.3), however, all gums derived from *Acacia* origin are found to contain arabinose and galactose as their major constituents are called

arabinogalactan AG, *Acacia* gum are considered as arabinogalactan protein AGP (Fincher et al., 1983).

Table 1.3   Chemical analysis of some African *Acacia* species gum:

Species	Ash %	N%	S.O.R	(μ)	Mwx 10^6	E.W	Uronic acid%	Reference
A. oerfota	nd	0.20	+98	09.80	0.87	3030	07.00	Anderson (1976)
A. nilotica	1.54	0.02	+108	09.50	2.20	1890	09.00	Anderson (1976)
A. mellifera	2.90	1.45	-56	23.50	410	843	20.90	Anderson (1979)
A. karoo	0.56	0.13	+54	Nd	1.46	Nd	12.00	Anderson et al (1984)
A. kirbit	2.48	0.09	+54	08.00	0.21	1817	09.70	Anderson&Farquha(1979)
A. hockii	1.30	0.23	+91	13.00	Nd	521	34.00	Anderson et al (1984)
A.ehreberg.	3.10	0.09	-0.7	07.00	0.27	1060	17.00	Anderson et al (1984)
A.rubusta	1.50	2.80	+36	Ns	0.72	1660	09.00	Chrmus (1984)
A.sieberana	0.28	0.19	+103	12.00	0.14	1230	04.00	Anderson et al (1973)
A.acatechi	3.90	Nill	-30	Nd	0.40	Nd	03.30	Anderson &Soni(1988)
A.erubescen	4.00	1.08	-13	08.00	200	874	20.10	Anderson &Farquhan1979
A.fleckii	nd	0.58	-32	13.00	415	918	19.20	Anderson &Farquhan 1979
A.laeta	2.90	0.56	-42	20.70	725	1250	14.00	Anderson (1976,1977)
A.polyacantha	nd	0.37	-12	15.80	Nd	2020	09.00	Anderson (1986)

S.O.R = Specific Optical Rotation, E.W = Equivalent weight

1.13   Gum *Acacia* processing

Raw gum in the form of tears from *Acacia* trees contains impurities such as (tree bark, bug parts, sand, dirt, etc.) which must be removed prior to use. The gum is processed to remove the foreign matter and to reduce the variability in quality. There are three types of gum processing.

1.13.1 Kibbled or granular gum

Kibbling is a mechanical grinding technique which breaks up gum nodules into smaller fraction of various specific sizes. The advantage of this processes no heat treatment and, therefore, the highest possible functionality; gum cannot be adulterated with other starch, sugar, etc. The disadvantages are gum can be adulterated with other gum, highly variability in quality, contains high foreign matter, high microbiology, high variable moisture, and slow in process dissolution.

1.13.2 Mechanical powder gum

The gum is milled to fine powder. The advantages, gum quality can be uniform as kibbled. The disadvantages is de adulterated with other gum.

1.13.3 Spray dried gum

In spry drying, the gum is kibbled (Williams, 1990) and dissolved in hot water, clarified by centrifugation and filtration, pasteurized to reduce microbiological content and enzymatic activity and subsequently spray dried. The advantages, gum quality can be extremely uniform with good process control, low microbiological, low moisture content, rabid dissolution. The disadvantages, as with any heat treatment, the drying process may affects the functionality of the gum.

1.14 *Acacia oerfota*

1.14.1 Botanical classification of *Acacia oerfota* tree

Kingdom: *plantae*

Division: *Magnoliophyt*

Class: *Magnoliopsida*

Order: *fabales*

Family: *Faba* Ceae

Sub family: *Mimosoideae*

Genus: *Acacia*

Species: *Acacia 9oerfota*

1.14.2 Synonyms

- Acacia virchowiana Vatke
- Acacia merkeri Harms.
- Acacia *oerfota*.

1.14.3 Common names

Laot (Sudan).

1.14.4 General description of *Acacia oerfota* tree

A somewhat obconical shrub, 1 to 5 m high, often with branches radiating from the base in all directions (Fig 1.5); branchlets grey-white, straight, stout; grey-white spines with brown tips, 0.5 to 1.5 cm long pinnae in 3 to 12 pairs, leaflets

in 5 to 15 pairs, about 0.3 cm long, Flowers in globose heads, off-white, very fragrant, though the bush often has an offensive smell. Pod pale yellow, longitudinally striate, distinctive, linear-elliptic, pointed at both ends, 5 to 10 cm long, 1.25 cm broad. Seeds olive-green, five to ten (Andrews, 1955).

Fig. 1.5 *Acacia oerfota* tree

1.14.5 The distribution

Acacia oerfota is found in Northern Africa: Egypt, North tropical Africa: Chad, Ethiopia, Sudan, East tropical Africa Kenya, Tanzania, Uganda, Asia temperate Oman, Sudia Arabia, Yemen, Western Asia, and Iran Fig 1.6 (USDA. 2008)
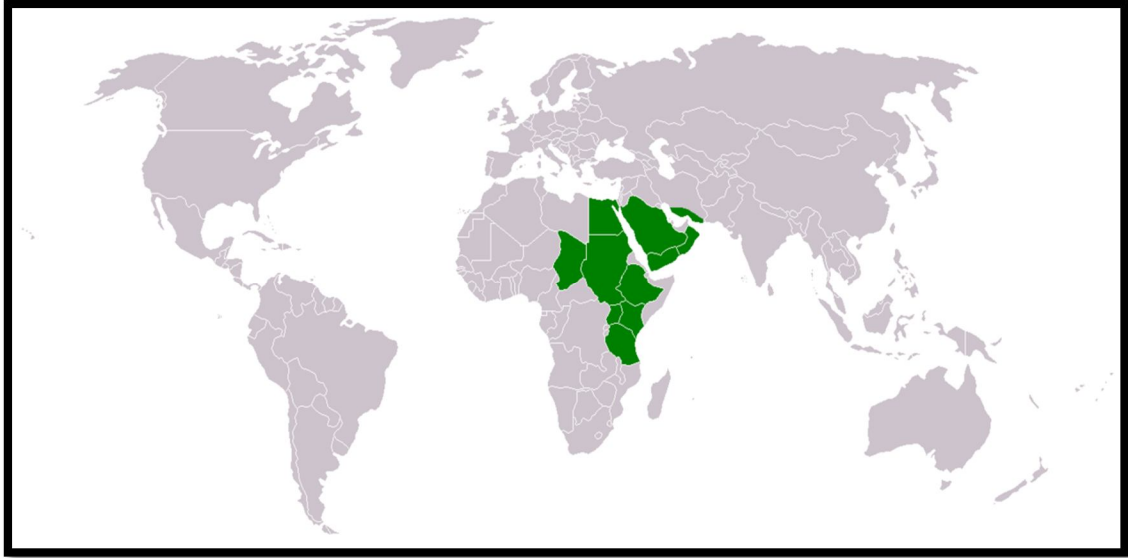
Fig. 1.6 World distribution of *A. oerfota* trees

1.14.6 Habitats

Semi- desert or dry savanna woodlands on dry hard clays, it also appears on denuded and over cultivated clay fields (Elamin, 1973)

1.14.7 Characteristics

An important browse shrub in the drier parts of Kenya; the leaves and pods are high in calcium, but the leaves have more phosphorus (Dougall , 1987).

1.14.8 Uses

Acacia oerfota is used as agent to treat body and joint pain and along with tonic serve the general purpose of making one feel better. The powder of the leave is used externally as a poultice to treat swelling. The root juice for scorpion bites (El-gazali, 1985). The smoke stems and branches is used to cure rheumatism and bachpains. The ash from the brunched plants is used as protection against anthron, while bark decoction taken as emetic (Gibreel, 2008)

1.14.9 *Acacia oerfota* gum

The gum vary from white through various shade of yellow, orange to dark brown. The highest grade sorts of gum *oerfota* are white, translucent and almost free of bark. The lower grades vary from light red to brown and may contain as much as very small amount of impurities (Fig 1.7). Natural gums are exuded in a variety of shapes and forms, usually the fragments are irregularly globular or tear shaped gum *oerfota* is exuded in the form of medium tears. The tears are then broken up into fragments.

Fig. 1.7 *Acacia oerfota* gum sample

1.14.10 Chemical composition of *Acacia oerfota* gum

The polysaccharide exuded by *Acacia oerfota* trees as a high, positive specific rotation, has low methoxyl and L-rhamnose contents, and contains D-galactose, L-arabinose, and D-glucuronic acid, which is present in two aldobiouronic acids, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and 4-*O*-(α-D-

glucopyranosyluronic acid-D-galactose. Autohydrolysis experiments gave 3-*O*-β-L-arabinofuranosyl-L-arabinose, 3-*O*-β-L-arabinopyranosyl-L-arabinose, β-(1 → 3)-linked-L-arabinose trisaccharides, and a degraded gum of a molecular weight 5,730, which was studied by linkage and methylation analysis. Partial hydrolysis with acid gave 3-*O*-β-D-galactopyranosyl-D-galactose and 6-*O*-β-D-galactopyranosyl-D-galactose. An examination of the *O*-methyl derivative of degraded gum *A* gave 2,3,4,6-tetra-, 2,3,4-, 2,3,6-, and 2,4,6-tri-, and 2,4-di-*O*-methyl-D-galactose; 2,3,4-tri-*O*-methyl-L-arabinose; and 2,3,4-tri-*O*-methyl-D-glucuronic acid. Degraded gum was subjected to a Smith degradation, and the product was examined by linkage and methylation analysis.

The *O*-methyl derivative of the whole gum gave 2,3,4- and 2,3,5-tri-, and 2,5- and 3,5-di-*O*-methyl-L-arabinose; 2,3,4,6-tetra-, 2,4,6-, 2,3,6-, and 2,3,4-tri-, 2,6- and 2,4-di-, and 2-*O*-methyl-D-galactose; and 2,3,4-tri-*O*-methyl-D-glucuronic acid. The whole gum was subjected to five successive Smith-degradations, and the Smith-degraded polysaccharides *S1–S5* were each examined by linkage and methylation analysis.

The structural evidence suggests that *A. oerfota* gum molecules possess highly branched D-galactan frameworks, to which are attached D-glucuronic acid residues and L-arabinose-containing side-chains, some of which are at least six units long. The gum contains the largest proportion of L-arabinose in any of the *Acacia* gum exudates studied to date (Anderson, D. M. W., 1968).

1.15 physico-chemical properties of *Acacia oerfota* gum

The identification of a particular gum from a series of different gum exudates needs an extensive number of analytical tests to perform. Table 1.4 shows the most important parameters that can be used to identify raw gum *oerfota* and distinguish it from other *Acacia* gums such as: (1) Specific optical rotation, (2) Nitrogen content, (3) Ash content, (4) Moisture content and (5) Absence of tannins. (Karamallah, 1999).

Table 1.4 Physico-chemical properties of *Acacia oerfota* gum

Property	Anderson	Karamallah (1999)	Ibrahim. A.T, (2006)
Moisture%	-	4.6	9.9 - 9.95
Ash%	1.54	1.03	1.45 - 1.5
Nitrogen%	0.2	0.35	0.1965
Protein%	1.32	2.19	1.2972
S.O.Rotation	+98	+64	+74
pH	-	3.5	4.633
Equi-weight	3030	4755	3125
Glucuronic acid	6.5	10.2	6.208
Intrinsic viscosity ml|g	9.8	-	5.6
M_w (g/mol)	0.87×10^6	-	4.5×10^6

1.15.1 Moisture content

Moisture content of the gum can be determined by measuring the weight lost after evaporation of water. Reducing the moisture content of the natural gum can be readily used as a tenable method of reducing the microbial counts. (Karamallah, 1999).

Table 1.5 Analytical data of the gum exudates from different *Acacia* species of the Sudan.

Species	Moisture (%)	Reference
A. senegal	12-15	Osman (1993)
A. seyal	8.5	Hassan (2000)
A. seyal var. *seyal*	11-16.1	Anderson *et al*., (1963)
A. seyal var. *fistula*	8.00	(Karamalla, 1999)
A .oerfota	**4.60**	**(Karamalla, 1999)**
A. sieberana var. *sieberana*	5.30	(Karamalla, 1999)
A. sieberana var .*vermesenii*	4.90	(Karamalla, 1999)
A. tortilis subsp. raddiana	4.40	(Karamalla, 1999)
A. tortilis subsp. spirocarpa	6.40	(Karamalla, 1999)
A. tortilis subsp. tortilis	6.10	(Karamalla, 1999)
A. drepandolobium	6.10	(Karamalla, 1999)
A. grrardii	5.90	(Karamalla, 1999)
A. ehrenbergiana	7.90	(Karamalla, 1999)
A. nilotica var nilotica	6.10	(Karamalla, 1999)
A. nilotica vartomentosa	5.80	(Karamalla, 1999)
A.nilotica varastringen	5.60	(Karamalla, 1999)
A. laeta	3.20	(Karamalla, 1999)
A. polyacantha	6.50	(Karamalla, 1999)

1.15.2 Ash content

The ash content indicates the presence of inorganic elements existing in salt form. Anderson *et al*., (1968) and Karamallah (1999) showed that the type of soil (clay or sand) affected the ash content significantly.

Table 1.6 Ash% of some African *Acacia* species gums.

Species	Ash (%)	Reference
A. senegal	2.87	Anderson (1977)
A. senegal	3.6	Osman (1993)
A. senegal	3.27	Omer (2006)
A. senegal	3.32	Abdelrahman (2008)
A. senegal	4.89	Younes (2009)
A. senegal	3.70	(Karamalla, 1999)
A. senegal	3.77	Karamallah *et al*., (1998)
A. senegal	3	. Jurasek *et al* (1993)
A. seyal	2.1	Hassan *et al*., (2005)
A. seyal	1.94 -3.55	Anderson *et al*., (1963)
A. seyal	3.93	Anderson (1977)
A. seyal	2.61	Omer (2006)
A. seyal	2.43	Abdelrahman (2008)
A. seyal	4.47	Younes (2009)
A. seyal var. *seyal*	2.30	(Karamalla, 1999)
A. seyal var. *fistula*	1.60	(Karamalla, 1999)
A. oerfota	**1.54**	**Anderson (1976)**
A .oerfota	**1.03**	**(Karamalla, 1999)**
A. fleckii	4.00	Anderson & Farquhan (1979)
A. tortilis subsp. raddiana	1.80	(Karamalla, 1999)
A. tortilis subsp. spirocarpa	2.03	(Karamalla, 1999)
A. tortilis subsp. tortilis	1.90	(Karamalla, 1999)
A. drepandolobium	0.01	(Karamalla, 1999)
A. grrardii	3.10	(Karamalla, 1999)
A. ehrenbergiana	2.60	(Karamalla, 1999)
A. nilotica subsp. nilotica	0.03	(Karamalla, 1999)
A. nilotica subsp .tomentosa	0.04	(Karamalla, 1999)
A.nilotica subsp. astringen	0.06	(Karamalla, 1999)
A. laeta	2.80	(Karamalla, 1999)
A. polyacantha	2.70	(Karamalla, 1999)
A.laeta	Nd	Anderson (1976, 1977)
A. mellifera	2.90	Anderson & Farquhan (1979)
A. sieberana var. *sieberana*	1.90	(Karamalla, 1999)
A. nilotica	2.48	Anderson (1976)

1.15.3 pH value

The hydrogen ion concentration plays an importance role in the chemistry and industry of gums. The change in the concentration of hydrogen ion may influence the solubility of gum or the precipitation of protein, therefore functional properties of a gum may be affected by *Acacia* change in pH, for example, viscosity and emulsifying power. Crude gums are slightly acidic because of the presence of free carboxyl groups of its constituent acidic residues, D-glucuronic acid and its 4-O-methy1 derivatives.

Table 1.7 pH of some African *Acacia* species gums.

Species	pH	Reference
A. senegal	4.66	Karamallah *et al.*, (1998)
A. senegal	4.66	Karamallah (1999)
A. senegal	4.78	Younes (2009)
A. seyal	4.2	Karamallah (1999)
A. seyal	5.16	Younes (2009)
A. sieberana var. *sieberana*	3.95	(Karamalla, 1999)
A. sieberana var .*vermesenii*	3.88	(Karamalla, 1999)
A .oerfota	**3.50**	**(Karamalla, 1999)**
A. drepandolobium	4.05	(Karamalla, 1999)
A. nilotica subsp. nilotica	4.10	(Karamalla, 1999)
A. nilotica subsp .tomentosa	4.48	(Karamalla, 1999)
A.nilotica subsp. astringen	3.75	(Karamalla, 1999)
A. polyacantha	4.25	(Karamalla, 1999)
A. seyal var. *seyal*	4.35	(Karamalla, 1999)
A. seyal var. *fistula*	3.80	(Karamalla, 1999)

1.15.4 Specific optical rotation

The optical activity of organic molecules (saccharides 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 (Stevens et al., 1977).

Table 1.8 Specific optical rotation of some African *Acacia* species gums.

Species	S.O.R	Reference
A. senegal	-30º	Anderson (1977)
A. senegal	-29º to -34.4º	Vavdevelde and Fenyo (1985)
A. senegal	-30.5º	Anderson (1991)
A. senegal	-20° to -32°	Jurasek et al, (1993)
A. senegal	-29º to -31º	Osman (1993)
A. senegal	-26º to -34º	Karamallah et al., (1999)
A. senegal	-27º to -36º	Idris et al., (1998)
A. senegal	-30.3º	Karamallah (1999)
A. senegal	-32º	Omer (2006)
A. senegal	-31.5º	Abdelrahman (2008)
A. senegal	-30º	Younes (2009)
A. senegal	-31.30	(Karamalla, 1999)
A. seyal	+51º	Anderson (1977)
A. seyal	+50.6º	(Karamalla, 1999)
A. seyal	+40º to +62º	Hassan (2000)
A. seyal	+53º	Hassan (2005)
A. seyal	+45º	Siddig et al., (2005)
A. seyal	+44º to +56º	Anderson et al., (1963)
A. seyal	+49.4º	Omer (2006)
A. seyal	+51º	Jurasek et al, (1993)
A. seyal	+61º	Abdelrahman (2008)
A. seyal var. fistula	+42.66	(Karamalla, 1999)
A. seyal var. seyal	+50.50	(Karamalla, 1999)
A. hockii	+91	Anderson et al., (1984)
A. nilotica	+108	Anderson (1976)
A. oerfota	**+98**	**Anderson (1976)**
A .oerfota	**+64.16**	**(Karamalla, 1999)**
A. rubusta	+36	Chrmus & Stephen (1984)
A. sieberana	+103	Anderson et al., (1973)
A. polyacantha	-12	Anderson (1986)
A. tortilis subsp. raddiana	+71.33	(Karamalla, 1999)
A. nilotica subsp. nilotica	+97.66	(Karamalla, 1999)
A. nilotica subsp .tomentosa	+80.16	(Karamalla, 1999)
A.nilotica subsp. astringen	+75.16	(Karamalla, 1999)

1.15.5 Cationic composition

The cations composition is amount of cations that incorporated in the gum. Snowden et al. (1987) carried out study on *A. senegal* cationic composition, and reported that 0.89%, 0.98% and 0.02% w/w of calcium, potassium and sodium ions respectively. Awad El Karim (1994) reported 0.45-0.85%, 1.0-1.6%, 0.18-0.36% and 0.53-0.099% of Calcium, potassium, magnesium and sodium respectively. Also Anderson et al. in 1992 and 1990 reported the values for some cations present in *A. senegal* gum.

Table 1.9 Cationic composition of gum arabic samples by Buffo et al.,(2001)

Mineral / Sample	*A. senegal* (ppm)	*A. seyal* (ppm)
P	2.46 – 6.51	12.83 – 24.04
K	6664 – 7735	2400 – 3558
Ca	5387 – 6314	9453 – 10145
Mg	1345 – 1987	1224 – 1295
Mn	2.37 – 8.76	0.72 – 2.61
Al	4.14 – 10.99	11.18 – 35
Fe	2.48 – 6.85	6.51 – 17.06
Na	3.84 – 11.99	6.54 – 49.55
Zn	0.24 – 0.38	0.26 – 0.33
Cu	1.14 – 1.45	0.21 – 2.49
B	0.49 – 0.76	0.52 – 0.63
Pb	< o.84	< 0.84
Ni	< 0.22	< 0.22
Cr	0.27 – 0.34	0.30 – 0.32
Cd	< 0.06	< 0.06

1.15.6 Viscosity

The viscosity of a liquid is its resistance to shearing, to 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). Viscosity can be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic viscosity (Table 1.10).

Table 1.10 Intrinsic viscosity of some African *Acacia* species gums

Species	Intrinsic viscosity cm^3g^-	Reference
A. senegal	13.4	Anderson (1977)
A. senegal	16.6	Karamallh, (1999)
A. senegal	21.8	Duvallet *et al.*, (1989)
A. senegal	13.4 -23.1	Jurasek *et al.*, (1993)
A. senegal	14.6	Omer (2006)
A. senegal	10.4 to 19.8	Idris *et al.*, (1998)
A. senegal	16.44	Karamallh *et al.*, (1998)
A. senegal	15.4	Abdelrahman (2008)
A. senegal	14.7 -17.3	Elmanan *et al.*, (2008),
A. senegal	18.9	Younes (2009)
A. seyal	11.9–17.6	Hassan *et al.*, (2005)
A. seyal	12.4	Jurasek *et al.*, (1993)
A. seyal	11.6 to 17.7	Flindt *et al.*, (2005),
A. seyal	14	Siddig *et al.*, (2005),
A. seyal	12.4	Anderson (1977)
A. seyal	11.4	Omer (2006)
A. seyal	11.6	Abdelrahman (2008)
A. seyal	11	Karamallh, (1999)
A. seyal	15.5	Younes (2009)
A. seyal	14.6 - 14.9	Elmanan *et al.*, (2008),
A.nilotica var.nilotica	10.13	Amera (2011)

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.

Relative viscosity (η_{rel}) is the ratio of the dynamic viscosity of the solution to that of the pure solvent ($\eta_{rel} = \eta/\eta_0$ where η is the dynamic viscosity of the solution and η_0 is the dynamic viscosity of the solvent). As it is a ratio, it is dimensionless having no units. It is related to the intrinsic viscosity $[\eta]$ by the Huggins-Kramer equation: $\ln(\eta_{rel})/c = [\eta] + k_2[\eta]^2c$, where c is the concentration.

Reduced viscosity (η_{red}) is the specific viscosity divided by the concentration. It has units of reciprocal concentration, for example, mL g^{-1}. It is related to the intrinsic viscosity $[\eta]$ by the Huggins-Kramer equation: $\eta red = [\eta] + k_1[\eta]^2c$, where c is the concentration.

Specific viscosity (η_{sp}) is one less than the relative viscosity ($\eta_{sp} = \eta_{rel} -1$; $\eta_{sp} = (\eta - \eta_0)/\eta_0$ where η is the dynamic viscosity of the solution and η_0 is the dynamic viscosity of the solvent). As with the relative viscosity, it has no units.

1.15.7 Calorific Value

The calorimeter C1 (Fig. 1.8) is used to determine the calorific value of solid and liquid materials according to national and international standards (DIN 51900, BS 1016 T5, ISO1928, ASTM 5468, ASTM 5865 and ASTM 4809)

Fig. 1.8 Calorimeter IKA C1 System and the accessories.

1.15.8 Nitrogen and protein content

The role of nitrogen and nitrogenous component in the structure, physicochemical properties and functionality of gum arabic was recently subjected to intensive investigation (Dickinson *et al.,* 1988, Randall *et al.,* 1989). 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 emulsifying stability.

Table 1.11 Nitrogen content of A. senegal and A. seyal

Species	Nitrogen content w/w	Reference
A. seyal var. seyal	0.09 – 0.19%	Anderson *et al*., (1963)
A. senegal var. senegal	0.31%	Osman (1993)
A. senegal var. senegal	0.33%	Karamallah *et al*., (1998)
A. senegal var. senegal	0.28 to 0.35%	Jurasek *et al*., (1993)
A. senegal var. senegal	0.29%	Anderson (1977)
A. senegal var. senegal	0.35%	Omer (2006)
A. senegal var. senegal	0.37%	Abdelrahman (2008)
A. seyal var. seyal	0.14%	Abdelrahman (2008)
A. seyal var. seyal	0.96%.	Hassan *et al*., (2005)
A. seyal var. seyal	0.15%	Siddig *et al*., (2005)
A. seyal var. seyal	0.14%.	Anderson (1977)
A. seyal var. seyal	0.14%	Omer (2006)
A. seyal var. seyal	0.14%	Jurasek *et al*., (1993)
A. seyal var. seyal	2.3%,	Younes (2009)

1.15.9 Equivalent weight and total uronic acid

Uronic acids are widely distributed in animal and plant tissues. They constitute a major component of many natural polysaccharides. Different methods have being developed for the determination of uronic acid in *Acacia gums*. These include colorimetric, decarboxylation and acid-base titrimetric methods (Ibrahim, 2006). The acid equivalent, or the titrable acidity, is determined as the mls of 0.02N sodium hydroxide that neutralizes 10 cm^3 of 3% w/v *Acacia gum* solution. Gums differ widely in their equivalent weight and uronic acid content. Karamalla in (1964) and Anderson (1976) reported that the equivalent weight of *Acacia oerfota* gum as 4755 and 3030 respectively.

1.15.10 Tannin content

One of the most important tests that can be used to identify gum *Acacia oerfota* and distinguish it from other *Acacia* gum is absence of tannins. Tannin content conducted by UV/Visible spectroscopy (Fig. 1.9).

A study had been done by Zahir (1998, cited by Karamallah, 1999) on raw gums from different *Acacia* species of Sudan-for their taxonomic classification, showed that these *Acacia* species could be divided into two main groups. Out of the thirteen gums tested, all but one fell into one group. The species falling in the large group showed presence of tannins in their gums. The tannin content ranged between 0.03 to 1.63%. The only gum that did not show presence of tannin was the gum from *A. senegal,* thus distinguishing itself distinctly and distantly from other *Acacia* gums. This finding was of significant importance when considering gums as food additives. It was established that tannins are anti-nutritional (Karamallah, 1999).
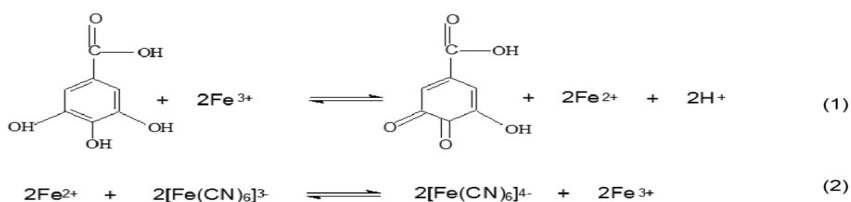

$$2Fe^{2+} + 2[Fe(CN)_6]^{3-} \rightleftharpoons 2[Fe(CN)_6]^{4-} + 2Fe^{3+} \qquad (2)$$

Fig 1.9 Perkin Elmer Lambda 40 UV/Vis spectroscopy

1.15.11 Number average molecular weight

An important group of absolute methods allowing the determination of the molecular weight of macromolecules is based on the measurement of colligative properties. Here, the activity of the solvent is measured in a polymer solution via determination of the osmotic pressure (π). The value of π required to determine the number-average molecular weight can be obtained using a membrane Osmometere.

1.15.12 Osmotic pressure

Osmosis is the phenomenon of penetration of a solvent into a solution through a semi permeable membrane. The tendency of solvent molecules to pass spontaneously into a solution, due to the inequality of chemical potential of pure solvent and solution estimated quantitatively by osmotic pressure, which has the dimension of pressure (atm). The osmotic pressure of a solution is equal to the additional pressure which must be applied to the solution to make the chemical potential of the component in solution equal to the chemical potential of the pure solvent (Billmeyer, 1971; Krigbaum and Flory, 1953). The Van't Hoff equation $\pi = C\, RT$ does not apply to polymer solutions, even though they are very dilute. The concentration dependence of osmotic pressure is expressed by a more complex equation which results if the concentration C is replaced by power series (Flory, 1953):

$$\frac{\pi}{C} = RT\left(\frac{1}{M_n} + A_2 C + A_3 C^2 + \cdots\right)$$

Π = osmotic pressure, A1 A2 = first and second virial coefficients, R = Gas constant

T is Temperature, C = Concentration

A_2 and $A_3 C^2$ is very small then:

$$The\ intercep = \frac{\pi}{C_{\to 0}} = \frac{RT}{M_n}$$

1.15.13 Infrared spectroscopy (FTIR)

Infrared spectroscopy is a useful chemical analysis tool in the study of carbohydrates. The useful range of an infrared spectrum is between 4000 and 625 cm $^{-1}$. When infrared radiation interacts with the matter it can be absorbed, causing the chemical bond in material to vibrate. Functional groups tend to absorb infrared radiation in the same wave number range regardless of the structure of the rest of the molecule (Perkin Elmer, 1994). There is a correlation between the wave numbers at which a molecule absorb infrared radiation and its structure. Any functional group has its characteristic vibration frequency, thus structures of unknown molecules can be identified from the infrared spectrum (Williams and Fleming, 1980; Smith, 1996).

1.16 The Rheology of *Acacia oerfota* gum

1.16.1 Introduction

Rheology is the science of flow and deformation of matter and describes the interrelation between force, deformation and time. Rheology is most sensitive method for material characterization, because flow behavior is responsive to properties such as molecular weight and molecular weight distribution (Wang, 2007).

Rheology is an applied science, and its aim is two fold: Firstly, rheologists try to understand the relation between structure and flow properties. This important for the intelligent design and/or formulation of materials for certain applications. Secondly, by studying the material behavior using simple deformations, fundamental relations will be derived between deformation and force. Thus we need Rheology to measure fluid properties, understand structure-property relations, model

behavior and to predict flow behavior of complex liquids under processing conditions (Bingham, 1920).

In principle, this definition includes everything that deals with flow, such as fluid dynamics, hydraulics, aeronautics and even solid state mechanics. However, in rheology we tend to focus on materials that have a deformation behavior in between that of liquids and solids, The term comes from The Greek philosopher Heraclitus described rheology as everything flows. Translated into rheological terms by *Marcus Reiner* this means everything will flow if you just wait long enough.

Fluid rheology is used to describe the consistency of different products, normally by the two components viscosity and elasticity. By viscosity is usually meant resistance to flow or thickness and by elasticity usually stickiness or structure. Rheology is applicable to all materials, from gases to solids. It was founded by two scientists meeting in the late '20s and finding out having the same need for describing fluid flow properties. The scientists were Professor *Marcus Reiner* and Professor *Eugene Bingham*. The science of rheology started in the 1920's when polymers started to be produced, leading to novel polymeric substances and new colloidal fluids (e.g. paints). Hence the Newtonian fluid and elastic solids are outside the scope of rheology and material behavior intermediate to these classical extremes will be studied here. The term "viscoelastic" is used to describe this behavior. Some fluids are however essentially inelastic, but have a viscosity which changes with the deformation state, they are called Non-Newtonian fluids (Braun *et al.*, 2000).

1.16.2 The quantities measured in rheology

In principle this is very simple - there are only three basic ideas. First: stress, the amount of force applied to a given area of the sample. Second: strain, the degree to which the material deforms. And third: the ratio of stress to strain, which defines the elastic modulus for a solid, and the ratio of stress to rate of strain (or flow rate), which defines the viscosity for a liquid. The big complication is that most materials, and especially all biological materials, have both liquid and solid aspects. Consequently, material properties like elastic modulus and viscosities are not constants but functions of time, force, the direction in which the force is applied, and so on (Mofrad MRK, 2006).

1.16.3 Classification of materials

Fluids are normally divided into three different groups according to their flow behavior: Newtonian fluids, Non-Newtonian fluids, time independent, and Non-Newtonian fluids, time dependent (Fig. 1.10).
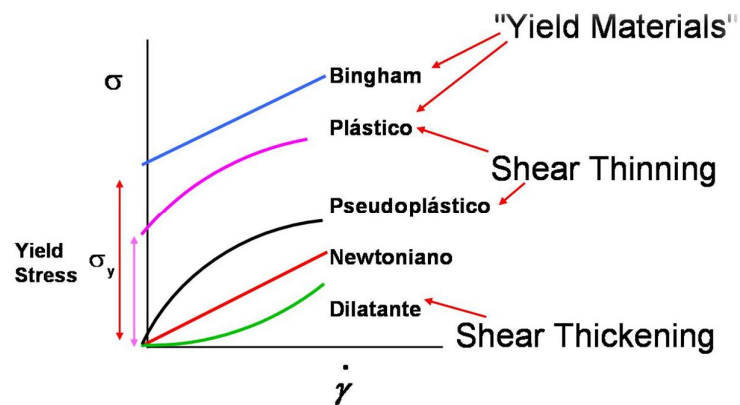

Fig. 1.10 Flow curves are normally use for the graphical description of flow behavior.

1.16.4 Stress and strain of the gum

Strain is what millions of people feel in their backs, or their relationships, and the cause of it is stress. Stress causes strain, but the amount of strain depends in some sense on how tough one is. Stress in rheology is, as one might expect, related to force. More precisely stress is the ratio of force to the area over which that force is exerted; it has units of force/distance^2. Bioengineering literature, stress is often given in cgs units of dyne/cm^2; in most other studies, stress is given in SI units of N/m^2, which is the same as a Pascal (1 Pa = 1 N/m^2 = 10 dyne/cm^2). Neither unit is particularly well scaled to cell biology, but 1 Pa = 1 pN/μm^2. There are different kinds of stress depending on the direction in which the force is exerted. A shear stress is parallel to the surface. For example, endothelial cells feel a shear stress on their apical surface due to blood flow in an artery, much as a river's edge is subjected to shear stress that depends on how fast a river flows. Strain in rheology is some what different from its everyday meaning: it's a purely geometrical quantity, a way to quantify the amount of deformation in a given material, and has no units. Much of the complexity of rheology relates to defining or measuring strain. Measurable quantities such as the distance by which some point moves in response to stress requires sometimes complicated formulas to convert them to strains, depending on the shape of the material and the place where the force is applied.The elastic modulus is the quantity that allows the prediction of how much a material will deform elastically when a certain amount of stress is applied, and viscosity is the analogous quantity that tells how fast the material will flow. The more a tissue is stressed, cell or protein network, the more it is strained. But the rate and degree to which it strains is, usually, a complex function of the magnitude of the stress and how long it is applied (Discher, et. al. 2005).

1.16.5 Viscosity and elasticity of the gum

The ratio of stress to strain is all what is needed to know the properties of the material. The difference between elastic and viscous is, basically, whether the strain reaches a limit or continuously increases in response to a constant stress. An ideal elastic material such as a spring deforms to a given strain in response to a given stress and then sits there forever unless the stress is removed, at which time the material returns to its initial shape. The elastic modulus, the ratio of stress to strain, is a constant in this case. All the work done by the initial stress (work = force × distance) was stored in the material (hence the term storage modulus, see below) and elastically recovered when the stress is removed. Elasticity in this context does not refer to whether a material is `stretchable' or not, but whether it returns to its initial shape when you stop pulling or pushing on it. There are different kinds of elastic modulus depending on the kind of stress. If it's a shear stress, then the ratio of stress to strain is the shear modulus. If it's elongational or compressional stress, then the ratio of stress to strain is generally called the *Young's* modulus, named for *Thomas Young* who also identified the cause of astigmatism. If a simple material conserves volume, then shear and Young's modulus are related by a factor of three. An ideal viscous material changes strain in proportion to the time that the stress is applied. In this case, the ratio of stress to the rate of strain defines the viscosity (Mothe` , C. G., & Rao, M. A.,1999).

1.16.6 The Viscoelasticity

As noted above, most materials are both viscous and elastic. The shear modulus and viscosities that define them depend on strain rates as well as strain magnitudes. Differentiating elastic from viscous effects requires measurements at different time scales and is usually done by performing

oscillatory deformations at different frequencies. As a result, data are often reported in terms of storage (elastic) or loss (viscous) modulus. The time dependence is important.

1.16.7 Kinematic and dynamic viscosity

Kinematic viscosity is measured with kinematic instruments, normally different types of cups which means that the knowledge and control of shear rates is limited or non-existent. Therefore kinematic viscosity values are of little or no use for design of equipment for non-Newtonian fluids.

Dynamic viscosity takes into account the effect of shear rate and time and is therefore the only type of viscosity relevant for non-Newtonian design purposes. Dynamic viscosity is measured with dynamic instruments, either rotating (shearing) or oscillating (Heilbrunn L, et. al, 1956). An instrument only capable of measuring shearing viscosities is called a viscometer and the oscillating type is called a rheometer.

1.16.8 Basic constitutive equations

Various models for approximation of rheological data have been presented. One of the most widely spread models is the so-called power law for approximation of viscosity data. The main reason for the power law being so popular is that the shearing rheological behavior of a fluid is represented simply by a straight line in a log-log shear rate/shear stress graph. Another reason is that the shearing behavior of most fluids lends itself to a good approximation applying the power law (Table 1.12).

Table 1.12 Mathematical models for flow behavior

Newtonian	$\tau = K\gamma$
Shear thinning	$\tau = K\gamma^n$ (n<1)
Shear thickening	$\tau = K\gamma^n$ (n>1)
Bingham	$\tau = \tau_0 + \eta_p\gamma$
Herschel-Bulkley	$\tau = \tau_0 + K\gamma^n$
Casson	$\tau^{1/2} = \tau_0^{1/2} + \eta_\infty^{1/2}\gamma^{1/2}$
Sisko	$\eta = \eta_\infty + K\gamma^{n-1}$
Ellis	$\gamma = K_1\tau + K_2\tau^n$
Carreau	$\dfrac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = [1 + (\lambda\gamma)^2]^{(n-1)/2}$

*τ shear stress γ shear rate τ_0 yield stress η_∞ limiting viscosity.

1.16.9 Viscous and elastic modulus

Rheological measurements are normally performed in kinematic instruments in order to get quantitative results useful for design and development of products and process equipment. For design of products, e.g. in the food, cosmetic or paint industry, rheometric measurements are often performed to establish the elastic properties, such as gel strength and yield value, both important parameters affecting e.g. particle carrying ability and spreadability. For design of process equipment the properties during shearing of the product is of prime interest. Those properties are established in a normal viscosity measurement.

A rheometric measurement normally consists of a strain (deformation) or a stress analysis at a constant frequency (normally 1 Hz) combined with a frequency analysis, e.g. between 0.1 and 100 Hz. The strain sweep gives information of the elastic modulus G', the viscous modulus G" and the phase angle d. A large value of G ' in comparison of G " indicates pronounced (Fig. 1.11).

elastic (gel) properties of the product being analyzed. For such a product the phase angle is also small, e.g. 20º (a phase angle of 0º means a perfectly elastic material and a phase angle of 90º means a perfectly viscous material). The frequency sweep gives information about the gel strength where a large slope of the G ' curve indicates low strength and a small slope indicates high strength.
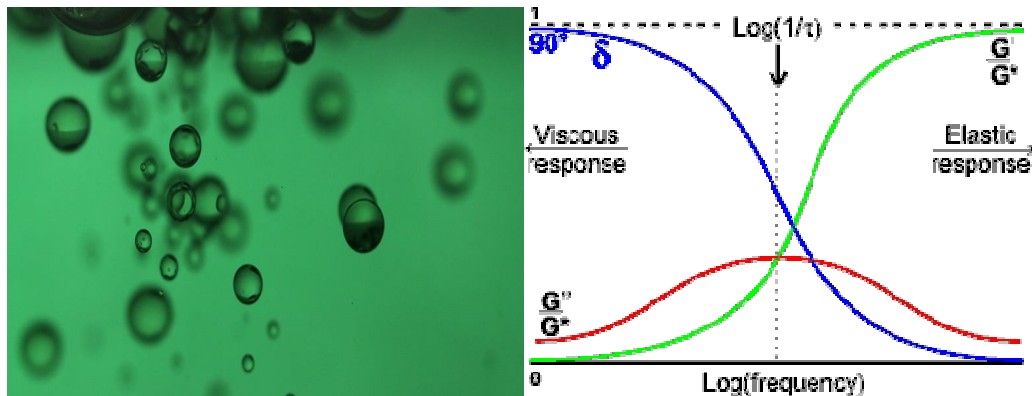

Fig. 1.11 The elastic modulus G', and the viscous modulus G''

A viscometric measurement normally consists of a shear rate analysis. The shear rate sweep should preferably cover the range applied in the intended equipment. For liquid foods a shear rate range from around 1 to 1,000 s-1 covers the needs for a low-viscous product.

1.16.10 Structural effects of the gum molecule

Linear and substantially linear polymers behave in a qualitatively predictable way with respect to the relationship of their viscosity to their structure and conformation. In dilute solutions this relationship depends effectively on the volume "swept out" (that is, the hydrodynamic volume) by the molecules as they tumble in the solution. At these low concentrations, where there is effectively no interaction between molecules and they are at their most extended, the viscosity may be little different from that of water; this small difference depending on the total

spherical volume (itself dependent on concentration and radius of gyration of the solute) taken up by the freely rotating molecules. The relationship between viscosities with concentration is generally linear up to viscosity values of about twice that of water (Barnes, et. al, 2001). This dependency means that more extended molecules increase the viscosity to greater extents at low concentrations than more compact molecules of similar molecular weight. Generally less-flexible links between sequential monomers in the polymeric chains give rise to more extended structures but the linkage spacing, direction and charge density are all important factors. Where residues are negatively charged, the repulsion between similar charges increases molecular extension but this can be reduced at higher ionic strength or below the pK_a's of the anionic groups and this reduction is particularly noticeable for polymers with high molecular mass. The lack of much change in viscosity of such molecules with ionic strength is indicative of an inflexible rod-type conformation. It should be noted that although attaching short sugar units as branch-points to linear polysaccharides does increase their rigidity into an extended structure, this is at the cost of greatly increased molecular mass. The extended nature of the molecules has an extreme effect on the molecular mass dependency of the viscosity as the hydrodynamic volumes (and hence viscosities) of compact (highly flexible but poorly hydrated) molecules increase approximately as the cube root of their molecular mass whereas those of more-extended well hydrated molecules (such as alginate and xanthan gum) increase approximately linearly with molecular mass. The relationship between the intrinsic viscosity $[\eta]$ and the relative molecular mass (M_w) is given by $[\eta] = K\ M_w^a$, the Mark-Houwink equation where K and a are constants. Amylose, carboxymethylcellulose, arabinoxylans and guar all

have exponents (a) of about 0.7. Knowledge of these constants allows the viscosity-averaged molecular mass to be calculated from viscosity data.

The viscosity increases with concentration until the shape of the volume occupied by these molecules becomes elongated under stress causing some overlap between molecules and a consequent reduction in the overall molecular volume with the resultant effect of reducing the amount that viscosity increases with concentration (under stress).
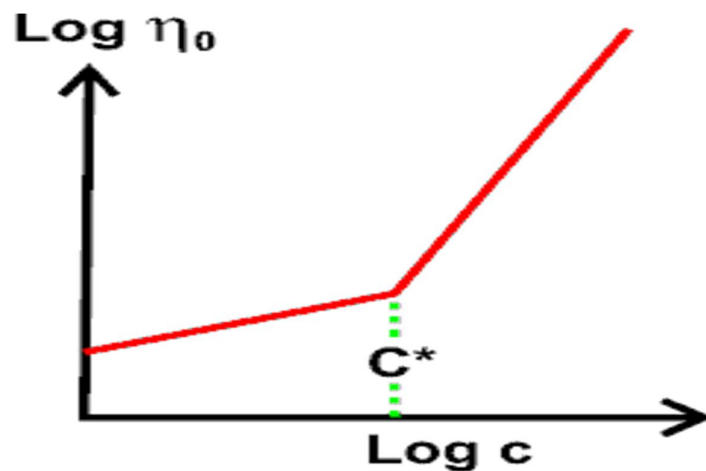

Fig. 1.12 A plot of log η_0 vs. log C

At higher concentrations (above a critical concentration C*) all the polymer molecules in the solution effectively overlap (Fig. 1.12), interpenetrate and become entangled (that is, their total hydrodynamic volume appears greater than the solution volume) even without being stressed, so changing the solution behavior from mainly viscous to mainly elastic with the viscosity (η_0 at zero stress) being mainly governed by the mobility of the polymer molecules. C* will depend on the shear strain rate as, at high shear strain rate, the molecules take up a less voluminous shape. At higher concentrations the viscosity increases up to about the fifth power of the concentration and this can cause apparently

synergic behavior of hydrocolloid mixtures, particularly if they cause phase separation with its inherent concentration increases.

At high shear strain rate (and sufficient concentration) molecules may become more ordered and elastic. Shear flow (and its related stress) causes molecules to become stretched and compressed (at right angle to stretch) resulting in isotropic solutions becoming anisotropic. After release from such conditions, the molecules relax back with time (the relaxation time). At low concentrations below the critical value (C*), the shear modulus of hydrocolloid solutions is mainly determined by the loss modulus at low frequencies (that is, G'' is relatively high for viscous materials). As G'' depends on the frequency but G' depends on the square of the frequency, G' becomes more important at higher frequencies. At higher concentrations in viscous solutions G' is generally greater than G'' throughout a wide frequency range. This difference is very large for strong gels when the frequency has almost negligible effect (that is, G' is high for elastic materials). Such gels often form above another critical concentration specific to the hydrocolloid, where junction zones occur so stabilizing intermolecular associations (Li, X., Fang, (2009).

1.16.11 Further rheological terminology

Dilatancy (**shear thickening**) shows an increase in viscosity with shear stress and strain due to structural enhancement. An example is uncooked corn starch paste where shear stress squeezes the water from between the starch granules allowing them to grind against each other. This property is often used in sauces where, for example, tomato sauce flow is prevented under small shear stress but then catastrophically fails, producing too great a flow, under greater stress (shaking). Another (and the strictly correct usage for the term) meaning for dilatancy concerns the increase in volume of suspensions of irregular particles with shear due to

the creation of small but empty cavities between the particles as they scrape past each other.

Eutectic point is the lowest possible melting point (equilibrium freezing point) that a mixture of solutes may have. No other composition of the same materials will have a lower melting point. Thin films of fluid may remain below the eutectic point in microcrystalline ice due to surface effects.

Fluidity is the reciprocal of the viscosity (= $1/\eta$).

Stoke (St) is a unit of kinematic viscosity ($cm^2\,s^{-1}$). The <u>SI</u> unit of kinematic viscosity is $m^2\,s^{-1}$ (= 10000 stoke).

Thixotropic liquids exhibit a time-dependent response to shear strain rate over a longer period than that associated with changes in the shear strain rate. They may liquefy on being shaken and then solidify (or not) when this has stopped.

At moderate concentrations above a critical value hydrocolloid solutions exhibit non-Newtonian behavior where their viscosity depends on the shear strain rate, typically as opposite, where γ is the shear strain rate, η_0 and η_∞ are the viscosities at zero and infinite shear strain rate respectively and τ is a shear-dependent time constant that represents the reciprocal of the shear strain rate required to halve the viscosity.

Tan (δ) = G''/G' where tan (δ) quantifies the balance between energy loss and storage. As tan ($45°$) $=1$, a value for tan (δ) greater than unity indicates more "liquid" properties, whereas one lower than unity means more "solid" properties, regardless of the viscosity. The shear modulus (resulting from changing strain) is the ratio of the shear stress to the shear strain.

1.16.12 The goal of the scientist, engineer, or technician on rheology

• To understand the kinds of flow and deformation effects exhibited by complex systems.

• To apply qualitative rheological knowledge to diagnostic, design, or optimization problems.

• To in diagnostic, design, or optimization problems, use or devise quantitative analytical tools that correctly capture rheological effects

Table 1.13 Process shear, shear rate, and applications from Barnes (1989)

Process shear	rate [s^{-1}]	Applications
Sedimentation	10^{-6} -10^{4}	Medicines, Paints,…
Leveling	10^{-3}-10^{-1}	Paints, inks
Draining under gravity	10^{-1}	Emptying tanks
Extrusion	10^{1}-10^{2}	Polymer melts, Dough
Chewing, Swallowing	10^{1} -1^{02}	Food
Dip Coating	10^{1} -10^{2}	Paints, confectionery
Mixing and stirring	10^{1}-10^{3}	Manufacturing liquids
Pipe flow	10^{2}-10^{3}	Pumping, blood flow
Spraying, brushing	10^{3} -10^{4}	atomization, painting
Rubbing	10^{4} -10^{5}	Skin cream and Lotion
Injection molding	10^{2} -10^{5}	Polymer melts
MILLING	10^{3}-10^{5}	inks, coatings
coating flows	10^{5} -10^{6}	coating flows
Lubrication	10^{3}-10^{7}	Engines

1.17 Molecular weight distribution of *Acacia oerfota* gum

1.17.1 Introduction

The molecular weight and molecular weight distribution of *acacia oerfota* gum was studded using Gel Permeable Chromatography (GPC) with different detectors such as Light scattering (LS), Refractive index (RI) and Ultra violet (UV).

Gel Permeable chromatography (GPC) is a type of size exclusion chromatography (SEC) that separates analytes on the basis of size. The technique is often used for the analysis of polymers. As a technique, SEC was first developed in 1955 by Lathe and Ruthven Lathe. The term gel permeation chromatography can be traced back to J.C. Moore of the Dow Chemical Company who investigated the technique in 1964 and the proprietary column technology was licensed to Waters Corporation, who subsequently commercialized this technology in 1964. GPC systems and consumables are now also available from a number of manufacturers. It is often necessary to separate polymers, both to analyze them as well as to purify the desired product (Moore, J.C., 1964).

When characterizing polymers, it is important to consider the Polydispersity index (PDI) as well the molecular weight. Polymers can be characterized by a variety of definitions for molecular weight including the number average molecular weight (M_n), the weight average molecular weight, the size average molecular weight (M_z), or the viscosity average molecular weight (M_v). GPC allows for the determination of PDI as well as M_v and based on other data, the M_n, M_w, and M_z can be determined.

1.17.2 Radius of gyration

Radius of gyration (R_g) is defined as the distance (r_i) between all pairs of entities (n). Where (n) is the number of entities in the chain and (r_i) is the radius of each from the center of mass (Fig. 1.13).

GPC separates based on the size or hydrodynamic volume (radius of gyration) of the analytes. This differs from other separation techniques which depend upon chemical or physical interactions to separate analytes (Skoog, D.A., 2006). Separation occurs via the use of porous beads packed in a column.
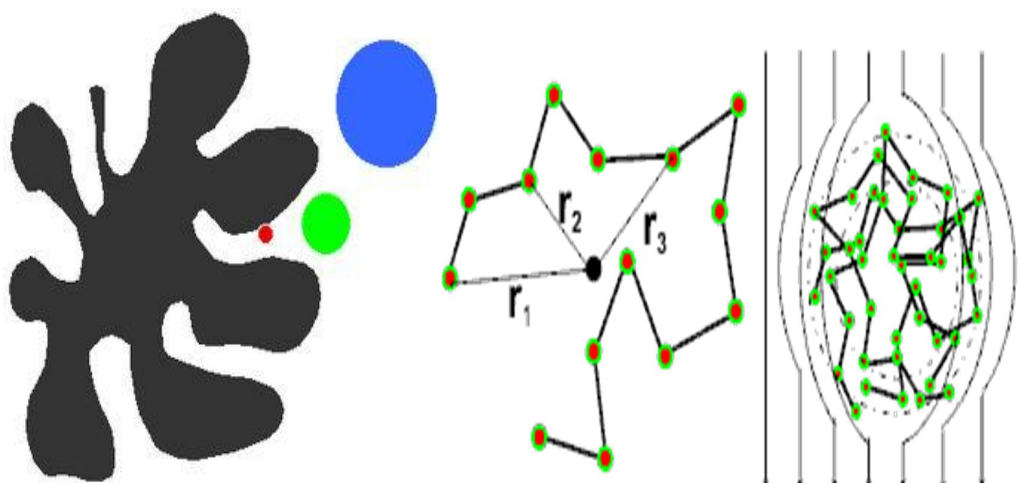
Fig. 1.13 Schematic of pore vs. analyte size and the radius of gyration

The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. These smaller molecules spend more time in the column and therefore will elute last. Conversely, larger analytes spend little if any time in the pores and are eluted quickly. All columns have a range of molecular weights that can be separated.

If an analyte is either too large or too small, it will be either not retained or completely retained, respectively. Analytes that are not retained are

eluted with the free volume outside of the particles (Vo), while analytes that are completely retained are eluted with volume of solvent held in the pores (Vi). The total volume can be considered by the following equation, where Vg is the volume of the polymer gel and Vt is the total volume.

$$V_t = V_g + V_i + V_o$$

As can be inferred, there is a limited range of molecular weights that can be separated by each column and therefore the size of the pores for the packing should be chosen according to the range of molecular weight of analytes to be separated. For polymer separations the pore sizes should be on the order of the polymers being analyzed. If a sample has a broad molecular weight range it may be necessary to use several GPC columns in tandem with one another to fully resolve the sample.

Typically for a linear polysaccharide (M_w 10^6) the Radius of gyration would be approximately 6 nm if spherically packed, 940 nm if as an extended stiff rod, and 17 nm if as a random coil. Its relationship to the effective radius the tumbling molecule represents to a flowing liquid (hydrodynamic radius, R_h) depends upon the flexibility and density of the structure; R_g/R_h generally being about 1.6 but lower for branched and globular structures and gels.

1.17.3 Light scattering

If a beam passes through of suspended particles the light is scattered; this phenomenon is called Rayleigh scattering. From the theory of scattering we can calculate the molar mass of the particle and obtain some idea about its shape. First, we consider the scattering by a particle that is small compared to the wavelength of the light. The monochromatic light beam passed into cell and the intensity of scattered light measured as a function

of the scattered angle and as a function of the wave length. The filter selects the wavelength to be used; the detector can be moved through an angle centered on the 90 degree position so that any dissymmetry can be measured. The scattering for large particles is not symmetric about the 0 degree angle (G.H.; Ruthven, C.R.J., 1956).

Whenever an electrical charge is accelerated, it radiate energy. If the oscillating electric field of a light beam act on a charge, the charge oscillates, is accelerated, and radiates a light beam of the same frequency. This oscillating charge is equivalent to an oscillating dipole moment, a charge q displaced through a distance x.

1.17.4 Rayleigh scattering

Scattering is a good approximation to the manner in which light scattering occurs within various media for which scattering particles have a small size parameter. In a typical Rayleigh scattering experiment, a well collimated, single frequency polarized light beam (e.g. from a laser) is used to illuminate a solution containing a suspension of the macromolecules of interest. The electric field of the polarized light beam is generally produced perpendicular to the plane in which the intensity and angular dependence of the subsequently scattered light is to be measured. The intensity carries information about the molar mass, while the angular dependence carries information about the size of the macromolecule. Light scattering is a non-invasive technique for characterizing macromolecules and a wide range of particles in solution. In contrast to most methods for characterization, it does not require outside calibration standards. In this sense it is an absolute technique. There are two different types of light scattering measurements for absolute molecular characterization:

Absolute molecular weights can be determined also via mass spectrometry, membrane osmometry, and sedimentation equilibrium (analytical centrifugation), only light scattering covers so broad a range of macromolecules including their oligomeric states. Most importantly, light scattering permits measurement of the solution properties of macromolecules. While a sedimentation equilibrium run may require 72 hours, a size exclusion chromatography/light scattering study may be completed in well under an hour and a batch mode analysis in a few minutes (Billmeyer, F. W. (1971).

1.17.5 Scattered Light and Molar Mass

The intensity of the radiated light depends on the magnitude of the dipole induced in the macromolecule. The more polarizable the macromolecule, the larger the induced dipole, and hence, the greater the intensity of the scattered light. Therefore, in order to characterize the scattering from a solution of such macromolecules, it is first necessary to know their polarizability. This may be determined from a measurement of the change, *dn*, of the solution's refractive index *n* with the molecular concentration change, *dc*, by measuring the *dn/dc*. When there are many macromolecules in solution, each macromolecule scatters light via the aforementioned induced dipole mechanism.

During the time of passage of the incident light wave front over the macromolecule, each element scatters in phase with the scattering of adjacent elements. Thus the scattered waves will add destructively or constructively producing constructive or destructive interference in certain directions. If the angular dependence of the scattered light is measured, it is possible to determine the size of the molecule. Here, the size measurement is known as the root mean square (rms) radius, or sometimes the "radius of gyration". The latter term is a misnomer since it describes a kinematic measure of a molecule rotating about a particular axis in space. The rms radius, on the other hand is a measure of its size weighted by the mass distribution about its

center of mass. Once the molecule's conformation is determined, (e.g., random coil, sphere, or rod), the rms radius can be related to its geometrical dimensions. For a sample containing a broad distribution of molecular masses, following separation by chromatographic means, the measured rms radius may be plotted against the correspondingly measured molar mass to determine the sample's conformation (Billmeyer, F. W. (1971).

1.17.6 GPC instrument

GPC is often used to determine the relative molecular weight of polymer samples as well as the distribution of molecular weights. What GPC truly measures is the molecular volume and shape function as defined by the intrinsic viscosity. If comparable standards are used, this relative data can be used to determine molecular weights within ± 5% accuracy.
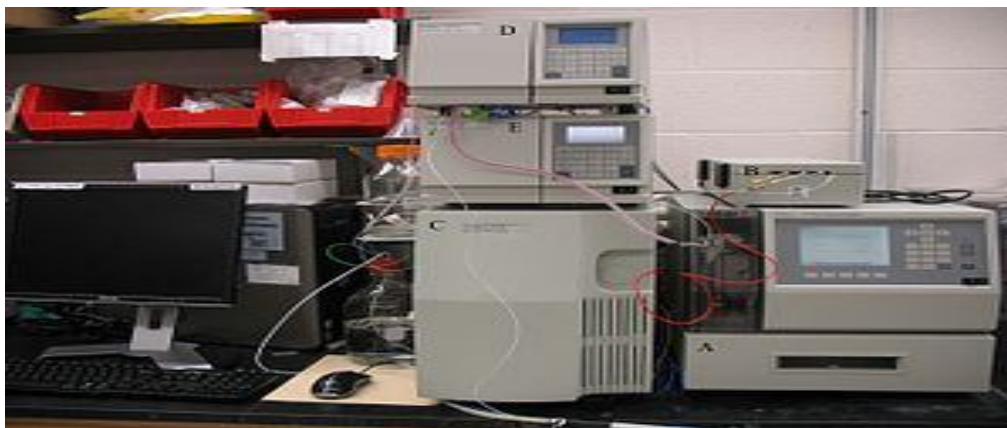

Fig. 1.14 A typical GPC instrument including: Auto sampler, Column, Pump, RI detector, and UV-vs detector

Gel permeation chromatography is conducted almost exclusively in chromatography columns. The experimental design is not much different from other techniques of liquid chromatography (Fig. 1.14). Samples are dissolved in an appropriate solvent, in the case of GPC these tend to be organic solvents and after filtering the solution it is injected onto a

column. The separation of multi-component mixture takes place in the column. The constant supply of fresh eluent to the column is accomplished by the use of a pump. Since most analytes are not visible to the naked eye a detector is needed. Often multiple detectors are used to gain additional information about the polymer sample. The availability of a detector makes the fractionation convenient and accurate.

Gels are used as stationary phase for GPC. The pore size of a gel must be carefully controlled in order to be able to apply the gel to a given separation. Other desirable properties of the gel forming agent are the absence of ionizing groups and, in a given solvent, low affinity for the substances to be separated. Commercial gels like PLgel, Sephadex, Bio-Gel (cross-linked polyacrylamide), agarose gel and Styragel are often used based on different separation requirements (Helmut, D. , 1969).

column used in GPC is filled with a microporous packing material. The column is filled with the gel. The eluent (mobile phase) should be a good solvent for the polymer, should permit high detector response from the polymer and should wet the packing surface. The most common eluents in for polymers that dissolve at room temperature GPC are tetrahydrofuran (THF), o-dichlorobenzene and trichlorobenzene at 130–150 °C for crystalline polyalkynes and m-cresol and o-chlorophenol at 90 °C for crystalline condensation polymers such as polyamides and polyesters.

There are two types of pumps available for uniform delivery of relatively small liquid volumes for GPC: piston or peristaltic pumps.

In GPC, the concentration by weight of polymer in the eluting solvent may be monitored continuously with a detector. There are many detector

types available and they can be divided into two main categories. The first is concentration sensitive detectors which includes UV absorption, differential refractometer (DRI) or refractive index (RI) detectors, infrared (IR) absorption and density detectors. The second category is molecular weight sensitive detectors, which include low angle light scattering detectors (LALLS) and multi angle light scattering (MALLS) (Trathnigg, B., 1995). The resulting chromatogram is therefore a weight distribution of the polymer as a function of retention volume.
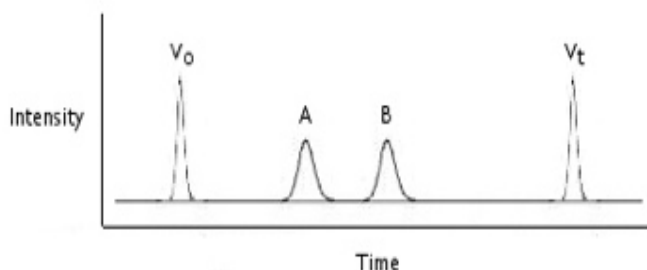
Fig. 1.15 GPC Chromatogram; Vo= no retention, Vt= complete retention, A and B = partial retention

The most sensitive detector is the differential UV photometer and the most common detector is the differential refractometer (DRI). When characterizing copolymer, it is necessary to have two detectors in series (Sandler, S.R.; Karo, W.; Bonesteel, J.; Pearce, E.M., 1998). For accurate determinations of copolymer composition at least two of those detectors should be concentration detectors (Trathnigg, B., 1995). The determination of most copolymer compositions is done using UV and RI detectors, although other combinations can be used (Pasch, H., 2000).

1.17.7 Advantages

As a separation technique GPC has many advantages. First of all, it has a well-defined separation time due to the fact that there is a final elution

volume for all unretained analytes. Additionally, GPC can provide narrow bands, although this aspect of GPC is more difficult for polymer samples that have broad ranges of molecular weights present. Finally, since the analytes do not interact chemically or physically with the column, there is a lower chance for analyte loss to occur. For investigating the properties of polymer samples in particular, GPC can be very advantageous. GPC provides a more convenient method of determining the molecular weights of polymers. In fact most samples can be thoroughly analyzed in an hour or less (Cowie, J.M.G.; Arrighi, V., 2008). Other methods used in the past were fractional extraction and fractional precipitation (Skoog, D.A., 2006).

1.17.8 Disadvantages

There are disadvantages to GPC, however. First, there is a limited number of peaks that can be resolved within the short time scale of the GPC run. Also, as a technique GPC requires around at least a 10% difference in molecular weight for a reasonable resolution of peaks to occur (Skoog, D.A., 2006). In regards to polymers, the molecular masses of most of the chains will be too close for the GPC separation to show anything more than broad peaks. Another disadvantage of GPC for polymers is that filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors. Although useful for protecting the instrument, there is the possibility of the pre-filtration of the sample removing higher molecular weight sample before it can be loaded on the column (Odian G., 1991).

1.17.9 GPC Fractionation

1.17.9.1 Arabinoglactan

Arabinogalactan protein is a broad term applied to diverse class of cell surface glycoproteins present in plant cell walls (Seifert et al 2007). It is heavily glycosylated, with only 2-10% comprising the protein region. The protein family has been earlier reported to contain O-linked glycans, whereas recent efforts employing mass spectrometry have revealed the presence of N-linked glycans as well within this protein family isolated from elongating cotton fiber cells (Kumar, et. al., 2013).

1.17.9.2 Arabinogalactan Protein

AGPs contains protein backbone of varied length (5-30kDa) with N-terminal secretory peptide followed by AGP, Fasciclin domains and a C-terminal glycosylphosphatidylinositol (GPI) lipid anchor site. In some plant cells, the length of the mature protein backbone is only 10-13 residues long and they are therefore called as Arabinogalactan peptides (Schultz et al 2000). The protein backbone contains domain rich in hydroxyproline/proline, serine, alanine and glycine amino acids. The repeated occurrence of Alanine/Serine/Threonine-Proline stretch (glycomodules) and the presence of hydroxyproline suggests the sites for O-linked glycosylation and arabinogalactan modification. The O-linked glycan chains predominantly contain galactose, arabinose, rhamnose, mannose, galacturonic acid and/or glucuronic acids and have a relatively high degree of polymerization, with a structure similar to arabinogalactan II (Mcneil et al 1984).

1.18 Emulsification Properties of *Acacia oerfota* Gum

1.18.1 Definition of emulsion

An emulsion is liquid preparation containing two immiscible liquids, one of which is dispersed as globules name as dispersed phase or internal phase in the other liquid name as continuous phase or external phase (Dickenson, 2003).

Dispersed phase ← [diagram] → Continuous phase

To stabilize these droplets, emulsifying agent should be added
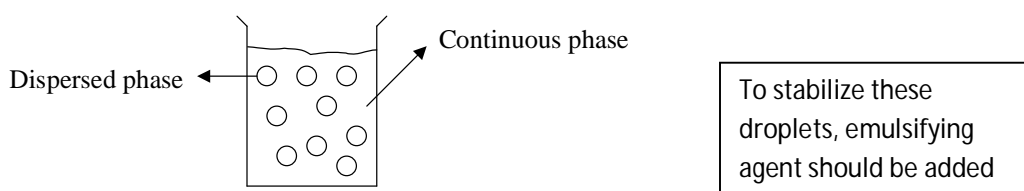
### 1.18.2 British Pharmacopoeia (BP) definition of oral emulsions

Oral emulsions are oral liquids containing one or more active ingredients. They are stabilized oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in oral emulsions. When issued for use, oral emulsions should be supplied in wide-mouthed bottles. Microemulsion has droplets size range 0.01 to 0.1 microns, but Macroemulsion has droplets size range approximately 5 microns (Dickenson, 2003).

1.18.3 The primary and secondary emulsion

Primary emulsion containing one internal phase, for example, oil-in-water emulsion (o/w) and water-in-oil emulsion (w/o). Secondary emulsion or multipleemulsion it contains two internal phase, for instance, o/w/o or w/o/w. It can be used to delay release or to increase the stability of the active compounds.

1.18.4 Theories of Emulsification

In case of two immiscible liquids as figure below the oil was separated from the water.

Separate rapidly into two clear defined layers
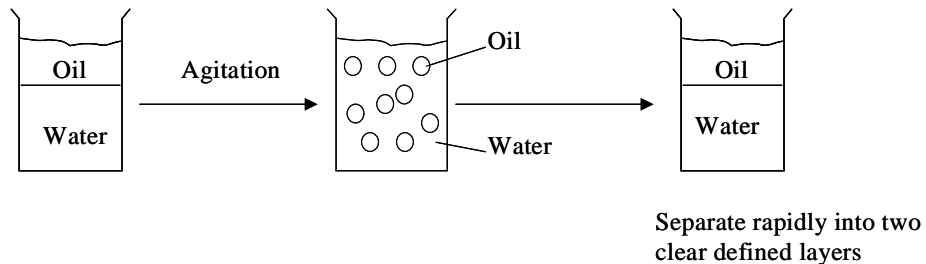
An explanation of this phenomenon is because of cohesive force between the molecules of each separate liquid exceeds adhesive force between two liquids. This is manifested as interfacial energy or tension at boundary between the liquids.

The producing of more small droplet in emulsion lead to increasing the surface area, increasing interfacial tension and the system thermodynamically unstable (high energy); and the system tend to separate in two layer to reduce the surface area. Therefore, to prevent the coalescence and separation, emulsifying agents have been used (McClements, 2007).

1.18.5 The emulsifying agents

The emulsifying agents defined as surface active agent adsorbed at oil/water interface to form monomolecular film to reduce the interfacial tension. Also emulsifying agents defined as hydrophilic colloids forming a multimolecular film around the dispersed droplet. Other definition of emulsifying agents is finely divided solid particles adsorbed at the interface between two immiscible liquid phases to form particulate film (Dickenson, 1992).

Table 1.14 The emulsifying agents

Emulsifying Agents	Examples
Carbohydrate Materials	Acacia, Tragacanth, Agar, Pectin.
Protein Substances	Gelatin, Egg yolk, Caesin
High Molecular Weight Alcohols	Stearyl Alcohol, Cetyl Alcohol, Glyceryl Mono stearate emulsion, cholesterol
Wetting Agents	Anionic, Cationic, Nonionic
Finely divided solids	Bentonite, Magnesium Hydroxide, Aluminum Hydroxide

## 1.18.6 Monomolecular adsorption

In emulsion, the surface area is high to maintain the dispersion of the droplets. Thus, based on the equation, surface free energy becomes high consequently. The only way to keep it low is to reduce the interfacial tension (Dickinson, 1992).

$$W = \gamma_{o/\omega} \cdot \triangle A$$

Surface free energy   Interfacial tension   Surface area

Surface active agent (SAA) is molecule which have two parts, one is hydrophilic and the other is hydrophobic.

Hydrophilic head

Hydrophobic tail

Water

Oil

Form monomolecular film
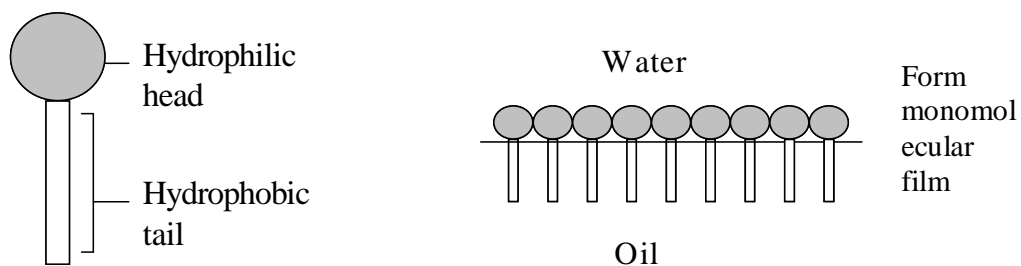
The functions of emulsifying agents is to provide stability to dispersed droplets are as following: Reduction of the interfacial tension by Forming coherent monolayer to prevent the coalescence of two droplet when they approach each other, or provide surface charge which cause repulsion between adjust particles (McClements, 2005).

Table 1.15 The Multimolecular adsorption examples

	Polysaccharides	Amphoterics	Synthetic or semi-synthetic polymers
colloids	Acacia Agar Alginic acid Carrageenan Guar gum Karraya gum Tragacanth	Gelatin	Carbomer resins Cellulose ethers Carboxymethyl chitin PEG-n (ethylene oxide polymer)

Other main function as emulsion stabilizers is by making coherent multi-molecular film. This film is strong and resists the coalescence. They have, also, an auxiliary effect by increasing the viscosity of dispersion medium. Most of the hydrophilic colloids form oil-in-water emulsions. Some of them can provide electrostatic repulsion like *acacia*, which contains Arabic acid and proteins (COOH and NH_3)

1.18.7 Solid particle adsorption

Finely divided solid particles are adsorbed at the surface of emulsion droplet to stabilize them. Those particles are wetted by both oil and water (but not dissolved) and the concentration of these particles form a particulate film that prevent the coalescence. Particles that are wetted preferentially by water from o/w emulsion, whereas those wetted more by oil form w/o emulsion. Note that they are very rare to use and can affect

rheology of the final product size of the particle is very important, larger particles can lead to coalescence.

1.18.8 The factors affecting the choice of emulsion type

The choice of emulsion depends on (1) properties and uses of final products (2) the other material required to be present.

- Oil-soluble drug is prepared in o/w emulsion due its solubility and its taste can be masked by adding flavoring agents
- For intravenous injection, o/w emulsion is the only type could be used.
- For intramuscular injection, both o/w and w/o types of emulsion could be used. Water-soluble drug can be prepared in w/o emulsion to get prolonged action (depot therapy)

Table 1.16 The differentiation between oil in water and water in oil emulsion

Oil in water emulsion	Water in oil emulsion
• For insoluble drug	• For water soluble drug
• For local effect	• Can be used to hydrate the upper layer of stratum corneum (moisturizing cream)
• Easily to wash from skin	• Can increase the absorption of drug from these formulation
• Doesn't have greasy texture of oily preparation	• Can be used to clean skin from dirt
• Acceptable by consumer	• Not acceptable by consumer

1.18.9 Emulsions Preparation Methods

1.18.9.1 Continental or Dry Gum Method

4 parts (volumes) of oil and 2 parts of water and one part of gum. *Acacia* or other o/w emulsifier is triturated with oil in a perfectly dry Wedgwood or porcelain mortar until thoroughly mixed. Glass mortar has too smooth a surface to produce the proper size reduction of the internal phase (Do not use glass mortar). After the oil and gum have been mixed, the two parts of water are then added all at once and the mixture is triturated immediately (Hunter, 1986).

1.18.9.2 English or wet Gum Method

Same proportion of oil, water and gum are used as in the continental or dry gum method but the order of mixing is different. Mucilage of the gum is prepared by triturating *acacia* (or other emulsifier) with water. The oil is then added slowly in portions, and the mixture is triturated to emulsify the oil. Should the mixture become too thick during the process, additional water may be blended into the mixture before another successive portion of oil is added.

1.18.9.3 Bottle or Forbes Bottle Method

Useful for extemporaneous preparation of emulsion from volatile oils or oleaginous substance of low viscosity. Put powdered *acacia* in a dry bottle add 2 parts of oil, then thoroughly shake the mixture in the capped bottle. A volume of water approximately equal to the oil is then added in portions, the mixture being thoroughly shaken after each addition. This method is not suitable for viscous oils (i.e. high viscosity oil) (McClements, 2005).

1.18.10 Control emulsion type during formulation

Volume of internal and external phases controls the type of emulsion. The smaller volume will be for the internal phase and the larger volume will be for external phase. In some cases, internal phases can be more than 50% of the total volume. The other control is Dominance of polar and non-polar characteristic of emulsifying agents (relative solubility of emulsifying agent in water and oil). Dominance of polar part results in formation of o/w emulsion and dominance of non-polar part results in formation of w/o emulsion. Note that polar groups are better barriers than non-polar; therefore, o/w emulsion can be prepared with more than 50 % of oil phase "internal phase".

1.18.11 Instability mechanisms of Emulsion

An emulsion is considered to be physically unstable if the internal phase tends to form aggregates of globules, or large globules or aggregates of globules rise to the top or fall to the bottom of the emulsion to form a concentrated layer of the internal phase. If all or part of the liquid of the internal phase becomes unemulsified on the top or bottom of the emulsion. Separation of the internal phase from the external phase is called breaking of the emulsion (Whitehurst, 2004).
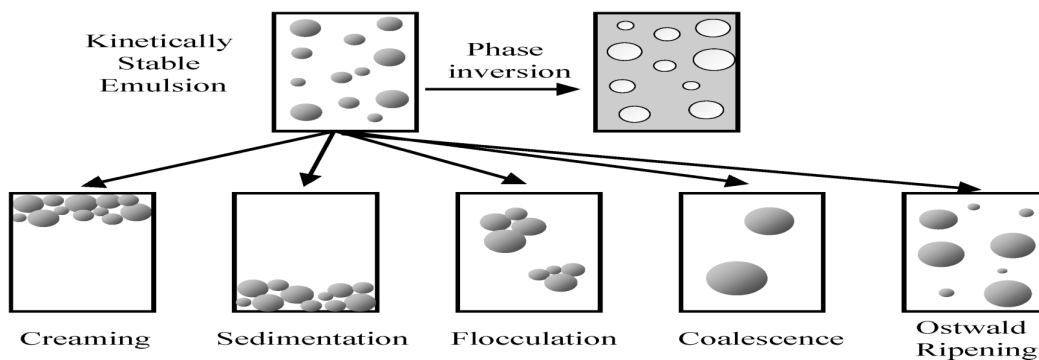

Figure 1.16 Schematic diagram of most common instability mechanisms

1.18.11.1 Phase Inversion

The relative volume of internal and external phases of an emulsion is important. Increase internal concentration increase viscosity up to a certain point. Viscosity will decrease after that point. At this point the emulsion has undergone inversion i.e. it has changed from an o/w to a w/o, or vice versa. In practice, emulsions may be prepared without inversion with as much as about 75% of the volume of the product being internal phase.

1.18.12 Applications of emulsions

The emulsions can be applied in many aspects such as food industry, mask the taste in many applications, convenient means of orally administration of water-insoluble liquids, facilitates the absorption of water-insoluble compounds, drugs, cosmetic and therapeutic uses (Dalgleish, 2001).

1.19 The objectives of this research

- To characterize *Acacia oerfota* gum by studying the general physicochemical properties of the gum.
- To compare and contrast the physicochemical properties of *Acacia oerfota* gum, with *Acacia Senegal* gum and *Acacia seyal* gum.
- To study:

  Rheological behavior of *Acacia oerfota* gum.

  Molecular weight and molecular weight distribution.

  Emulsification properties of *Acacia oerfota* gum.

Chapter Two

Materials and Methods

2.1 Sample collection

gum *oerfota* samples were collected from two locations Singa city Blue Nile state, and Wadel hadad Aljazeera state (15 sample of each location) during February 2015 (Table 2.1 and Fig 2.1). 10 samples of each location collected (sample/tree) and 5 sample collected (3tree/sample). Several *oerfota* trees were tapped by making incisions about 15cm long and 3 cm wide using an axe. 10 to 20 blazes were made on branches of the trees.

2.1.1 Sample code

Comp.1 prepared by mixing equal amount of (sample 1 - 4) Wadel hadad.

Comp.2 prepared by mixing equal amount of (sample 5-10) Wadel hadad.

Comp.3 prepared by mixing equal amount of (sample11-15)Wadel hadad.

Comp.4 prepared by mixing equal amount of (sample 1-15)Wadel hadad.

Comp.5 prepared by mixing equal amount of (sample 1-5) Senga.

Comp.6 prepared by mixing equal amount of (sample 6-10) Senga.

Comp.7 prepared by mixing equal amount of (sample 11-15) Senga.

Comp.8 prepared by mixing equal amount of (sample 1 - 15) Senga.

Table 2.1 Samples code and location of A. *oerfota* gum (Collection date February/2015)

| Wadel hadad location		Senga location	
No	Code	No	Code
1	sample.1	20	sample.16
2	sample.2	21	sample.17
3	sample.3	22	sample.18
4	sample.4	23	sample.19
5	sample.5	24	sample.20
6	sample.6	25	sample.21
7	sample.7	26	sample.22
8	sample.8	27	sample.23
9	sample.9	28	sample.24
10	sample.10	29	sample.25
11	sample.11	30	sample.26
12	sample.12	31	sample.27
13	sample.13	32	sample.28
14	sample.14	33	sample.29
15	sample.15	34	sample.30
16	Comp.1	35	Comp.5
17	Comp.2	36	Comp.6
18	Comp.3	37	Comp.7
19	Comp.4	38	Comp.8
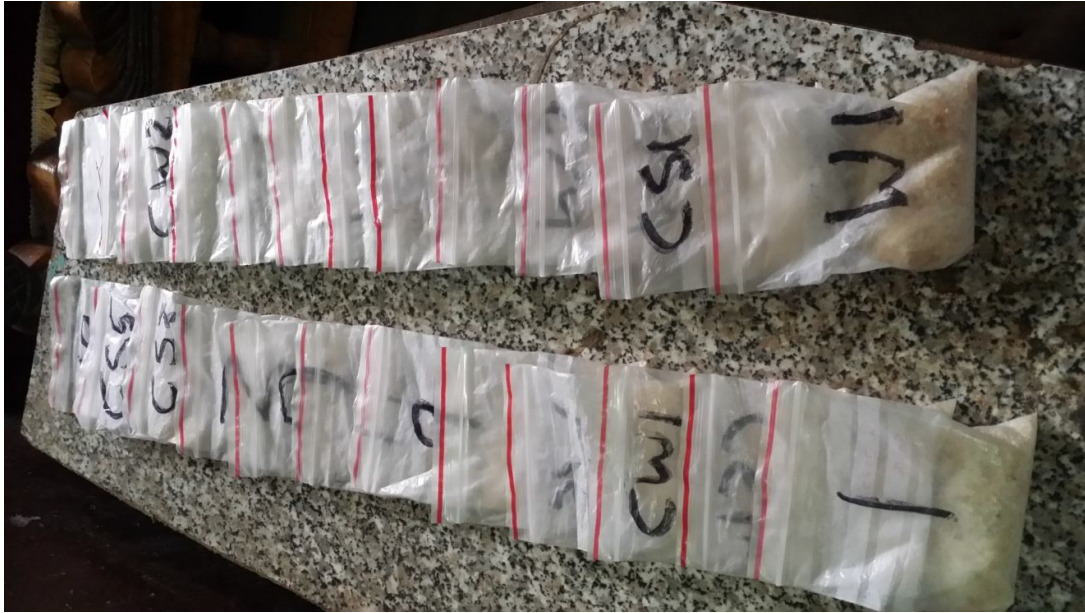
Fig. 2.1 Gum *oerfota* samples

2.2 Purification of gum samples

All of Gum samples were dried at room temperature and cleaned by hand to remove foreign particles. The samples were then ground using a mortar and pestle, and kept in containers for further analysis.

2.3 Determination of physico-chemical properties

2.3.1 Moisture content

Moisture content was determined according to FAO (1990). One gram of the ground gum was oven-dried to constant weight at 105 $C°$. The moisture content was calculated as the percentage of the loss in weight to the initial weight.

Moisture content %   = (Initial weight – final weight) × 100

                                       Initial weight

2.3.2 Total ash content

Total ash content was determined according to FAO (1990). One gram of gum sample placed in a crucible, and ignited in a muffle furnace at 550 $^{\circ}$ C for 5 hours. Total ash was calculated as follows:

$$\text{Total ash } (\%) = \frac{w_2 \times 100}{w_1}$$

Where: W_1= Original weight of the sample, W_2 = weight of the sample after ignition

2.3.3 pH

The pH was determined on 1% aqueous solution of gum using Berkman Zeromatic IV pH meter at room temperature.

2.3.4 Specific optical rotation

The observed optical rotation was determined for 1% aqueous gum solution using a Bellingham and Stanley polarimeter fitted with a sodium lamp and with a cell of path length of 20 cm at measurements, and the specific optical rotation was calculated according to the following equation:

Specific rotation $[\alpha]_D^T = [(\alpha \times 100)/(C \times L)]$.

Where:

α = observed optical rotation.

C = concentration of gum solution.

D = sodium immition line = 532 nm.

T = temperature.

L = length of polarimeter tube in decimeters.

2.3.5 Cationic composition

Cations composition of A. *oerfota* gum was determined by Electronic scanning microscope – EDX spectroscopy (Fig. 2.2), and the percentage of ions composition as the percentage of gum sample and as the percentage of total ions% was digital calculated.
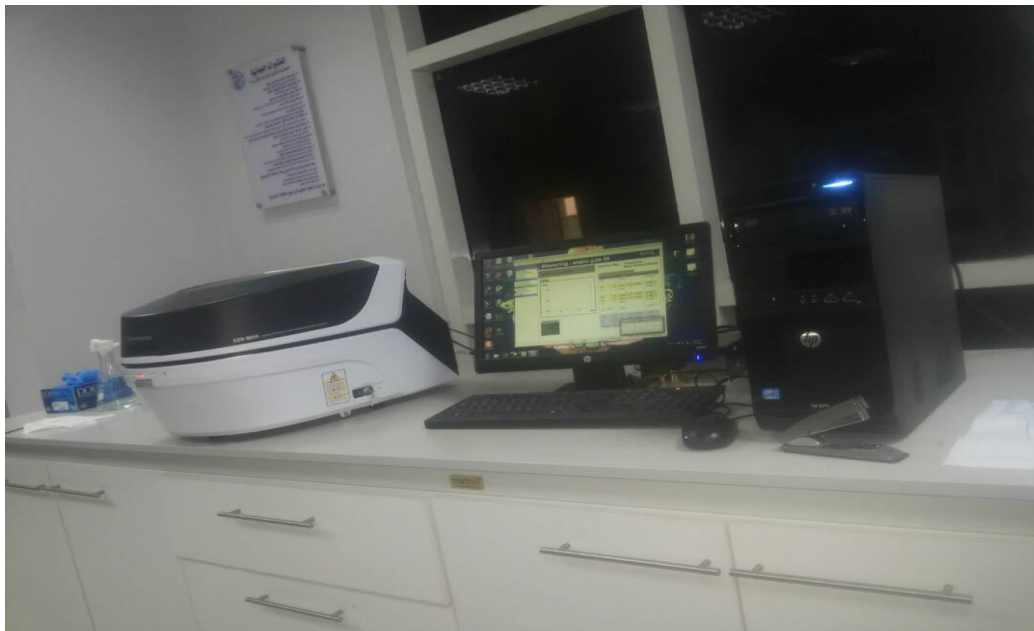

Fig. 2.2 Electronic scanner microscope - Energy dispersive X - Ray fluorescence spectrometer EDX - 7000/8000

2.3.6 Viscosity measurements

The viscosity was determined on gum solutions using U-tube viscometer immersed in a constant temperature water bath set at 25 C°. Gum solutions (0.5%, 1%, 2%, 3%, and 4%) was prepared in 0.2 M NaCl, then filtered and transferred into the viscometer. The intrinsic viscosity was calculated by:

Relative viscosity $[\eta_{rel}] = \eta/\eta_{\circ} = t/t_{\circ}$

Specific viscosity $[\eta_{sp}] = \eta_{rel} - 1$

Reduced viscosity $[\eta_{red}] = \eta_{sp}/C$

Intrinsic viscosity $[\eta] = \lim_{c \to 0} \eta_{sp}/C]$

The intrinsic viscosity $[\eta]$ is determined from the intercept in the plot of $\eta_{reduced}$ as a function of a sample concentration at zero concentration (infinite dilution). The inherent viscosity is determined from the intercept in the plot of η_{rel}/c as a function of a sample concentration at zero concentration (infinite dilution)

2.3.7 Calorific Value

The IKA C1 calorimeter system was calibrated by standard IKA C723 Benzoic acid tabs about 1g (2 Tabs), with cross cal.val. 26461J/g, RSD 0.03%, and LOT SZBD2180V.The temperature was 19 C, the gas pressure (Oxygen) was 30 bar, and the Pump flow 2700 rpm. Then weighted about 0.5g of A. *Oerfota* gum and placed into a small plastic bag which have cross value 46463j/g, the bag was covered by rolling and placed into a decomposition vessel which is surrounded by a water jacket. The sample was combusted in an oxygen atmosphere, and the calorific value of the sample was calculated.

2.3.8 Nitrogen and protein Content

The total nitrogen in gum samples was determine by used The Kjeldahl method according to AOAC (1990). The method consists of three basic steps: step one digestion of the samples in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia, step two distillation of the ammonia into a trapping solution, and step three quantification of the

ammonia by titration with a standard solution. The reactions involved in these steps can be shown as follows:

- Sample + H_2SO_4 (conc.) +Heat → $(NH_4)_2SO_4$
- $(NH_4)_2SO_4$ + 2 NaOH → $2NH_3$ + Na_2SO_4 + $2H_2O$
- NH_3 + H_3BO_3 → NH_4^+ + $H_2BO_3^-$
- $H_2BO_3^-$ + HCl → H_3BO_3 + Cl^-

The method

0.5 gram of each sample (in duplicate) was weighed and transferred to Kjeldahl digestion flasks and Kjeldahl tablet (copper sulphate-potassium sulphate catalyst) was added to each. 10 cm^3 concentrated, nitrogen free, sulphuric acid was added. The tube was then mounted in the digestion heating system which was previously set to 240ºC 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 attain room temperature. Their contents were 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 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.

$$\%N = \frac{14.01 \; x \; M \; x \; (volume \; of \; titrant - volume \; of \; blank) x \; 100}{weight \; of \; sample \; (grams)}$$

Where: M is the molarity of hydrochloric acid. Protein content was calculated using nitrogen conversion factor resulting from amino acid analysis as follows: % protein = % N x 6.51

2.3.9 Acid Equivalent weight

A glass column was packed with an Amberlite Resin IR (180H+). HCl 0.2M was passed through the column until the resin was thoroughly washed with the acid. Then this was followed by distilled water until the column was chloride free (20 cm³ eluent equalize to one drop 0.1N NaOH). 50 cm³ of 3.0% gum solution was passed through the column, followed by the distilled water until a volume of 250 ml of the eluent and washing were collected. This 250 cm³ of the eluent titrated against 0.1N NaOH. The apparent equivalent weight of the acid was calculated by:

$$Equivalent\ weight = \frac{weight\ of\ the\ sample\ \times\ 1000}{Volume\ of\ titer\ \times\ molarity\ of\ alkali}$$

2.3.10 Total Uronic acid

Uronic acid% was determined by multiplying the molecular weight of uronic acid (194) by 100 and dividing by the apparent equivalent weight of gum sample as follows:

$$Uronic\ acid\% \quad = \frac{194\ \times\ 100}{Eq.wt.}$$

2.3.11 Determination of Total Polyphenol (Tannin %)

The tannin content determined according to the Prussian blue assay originally devised by price and Butler and subsequently modified (Graham, 1992). Tannin content is taken her to represents the "total phenols" and more accurately the "Gallic acid equivalents" as Gallic acid – 99% in purity purchased from sigma Aldrich – was used as analytical standard for determining the hydrolysable tannins. 500 µg/g Gallic acid was prepared in distilled water. This was then serial diluted to obtain concentration of 400, 300, 200, 100, and 50 µg/g as standards. 0.10*ml* of each sample or standard

was dispensed in a 30*ml* universal. 3ml of distilled water was added fallowing by vortex mixing for 30sec. Next 1.00ml of 0.016*M* (0.526*g*/100*ml*,w/v) Potassium hexacyano ferrate(III) [*K₃[Fe(CN)₆]*], Fallowed by 1.00ml of 0.02(0.324*gFeCl₃*/100*ml* d.w+ 0.83*mlHCl*) Ferric Chloride (*FeCl₃*) were added and immediately mixed by vortex mixer 30sec. Exactly 15 min after adding the reagent to the sample 5.00ml of stabilizer was added and vortex mixed 30sec.The stabilizer was prepared by mixing 10.00ml of 85% phosphoric acid, (*H₃PO₃*), 1.00ml of 1% gum Arabic , and 30 ml of distilled water, then exactly after15 min The absorbance was read at 700 nm in triplicate for standard solutions, using (Perkin Elmer Lambda XLS+, UV/Vis spectroscopy). Solvent only blank were also prepared by adding all reagents and 0.1ml of solvent instead gum *Acacia oerfota* or Gallic acid standards. The absorbance was read at 700 nm in triplicate for all using Perkin Elmer Lambda 40 UV/Vis spectroscopy. The error in measuring the tannins content was below 10% for all samples and the average was taken.

2.3.12 Determination of number average molecular weight by Osmotic pressure

150 micro meter of different concentration (0.25%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) of A. *oerfota* gum samples was ejected into Osmometere 150 and the osmotic pressure was digital determined at 25 C^0. The number average molecular weight determined using equation:

$$The\ intercept = \frac{\pi}{C_{\to 0}} = \frac{RT}{M_n}$$

Π = osmotic pressure, R = Gas constant, T is Temperature, C = Concentration, M_n = Number average molecular weight.

2.3.13 Infrared spectroscopy (FTIR)

The Fourier Transform Infra-red (FTIR) spectra of gum samples were obtained on a Thermo-Nicolet-IR300 spectrophotometer using potassium bromide (KBr) pellets. The gum sample and the KBr, one gram each, were first ground in a mortar. Which was mounted in the instrument and the spectrum was then obtained.

2.4 Rheological measurement

The 50% w/w (based on dry weight) of gum *Acacia oerfota* solution were prepared in distill water, then the solution agitated on a tube roller mixer (SRT9. Stuart Scientific, UK) overnight to ensure that the sample fully dissolves and hydrated. The solutions were then centrifuged for 10 minutes at speed of 3000 rpm using (Megafuge 1.0R, Heraeus SEPATECH, Germany) centrifuge. One dilution was prepared from stock solution (25% w/w) and recentrifuged as previous procedure to investigate the rheological behavior. Rheological measurements were carried out using Malvern Series KINEXUS pro+ rheometer (Malvern Instruments Ltd., Malvern, Worcester, UK), fitted with cone and plate geometry with a cone diameter of 40 mm and an angle of 20. Steady shear viscosity curves were measured for gum solutions ranging from 10 to 50 % w/v both upon shear rate ramp-up (from 0.01 to 10000 s-1) and subsequent shear rate ramp-down (from 10000 back to 0.01 s-1). Dynamic rheological measurements, to determine the elastic modulus (G′), viscous modulus (G″) and dynamic viscosity, were performed in the frequency range of 0.1–10 Hz. The linear viscoelastic region was assessed, at 1 Hz. The temperature of the samples were controlled within 0.1 C using a Peltier element. The rheometer control and data processing was done by computer software.

Kinexus pro+

Research grade rheometer for complex fluids characterization

2.5 Molecular weight and molecular weight distribution

2.5.1 Sample preparation

2.0 mg/ml fallowed by one dilution (1.0 mg/ml) gum samples was prepared (based on dry weight) in 1mM phosphate buffer at pH 7 containing 0.2M NaCl, and hydrated by roller (SRT9. Stuart Scientific, UK) mixing the solution overnight to ensure that the sample fully dissolved and hydrated. The solutions were then centrifuged for 10 minutes at speed of 3000 rpm using Megafuge 1.0R (Heraeus SEPATECH, Germany) centrifuge. The gum solutions were then filtered using 0.45-µm nylon filter (Whatman, 13 mm) prior to injection into the GPC-MALLS system.

2.5.2 GPC-MALLS

The gum exudates from A. *oerfota* tree were subjected to average molecule weight M_w and molecular weight distribution study using different techniques such as Osmometere, gel permeation chromatography (GPC-MALLS). The system utilises Waters (Division of Millipore, USA) Solvent Delivery System Model 6000A connected to a column containing Superose 6 (Amersharm Biosciences) (10 x 300mm), manual Rheodyne Model 7125 syringe. The column eluent was

monitored by three detectors, refractive index (RI) Wyatt Optilab DSP interferometricrefractomter operated at 633 nm (Wyatt Technology Corporation, USA), multi-angle laser light scattering photometer DAWN EOS using He–Ne laser at 690 nm (Wyatt Technology Corporation, USA), and an Agilent 1100 series G1314A UV detector (214 nm, Agilent Technologies). RI provides an accurate concentration profile, MALLS enables absolute molecular mass and radius of gyration (Rg), and the UV detects the proteinaceous components of the gum. (Katayamaa*et al*., 2006). The data was processed by the Astra for Windows software (version 4.90.07, Wyatt Technology Corporation).

2.6 Emulsification properties of the gum

2.6.1 Emulsion preparation

Distilled water was added to 8 g of the gum sample (based on dry weight) in a glass bottle to give 40 g in total with a concentration of 20 % (w/w) gum solution. The sample was agitated on a roller mixer overnight until the sample is completely dissolved. Exact calculated grams for each samples (in the range from about 19.97 to about 20 g) of the prepared gum solution was filtered using 100 µm mesh then mixed with 0.52 ml of 10 % (W/V) sodium benzoate solution as a preservative, and 0.48 ml of 10 % (W/V) citric acid solution to adjust the pH to 4, 15.71 ml , and 15.73 ml of distilled water was added, then, 4.2 g of ODO oil (10%) was added to the gum solution to give a total of 40 g and final concentration of 10%. The mixed solution was homogenized for 3 minutes using a POLY TRON (PT 2100, KINEMA TICA AC) homogenizer at 22000 rpm (Fig. 2.3). Impeller (PTDA21 9 mm tip diameter) was used as dispersing tool. To achieve small particle size < 1 micron, the pre-emulsified mixture was homogenized using a high-pressure NanoVater

(NV30-FA, MITSUBISHI GOT1000.). In order to achieve effective disaggregation of the gum it was passed twice at 75 MPa. The final emulsion kept in closed glass universals, and emulsion particle size was measured. then putted at 60°C in the Vacuum Oven (GALLENKAMP. OVA031.XX1.5). Droplet size was remeasured after 3 and 7 days.

Fig. 2.3 POLY TRON (PT 2100, KINEMA TICA AC) homogenizer

2.6.2 Droplet size analysis

The droplet size distribution of the emulsions was measured, using Mastersizer 3000 (Fig. 2.4), a laser diffraction particle size analyzer (Malvern Instruments). Distilled water was used as dispersant and a value of 1.45 was used for the refractive index for oil phase (ODO). Emulsification stability of samples kept at 60C was evaluated by particle size change after accelerated stability test for 3 and 7 days. The particle size of the emulsions was described by the volume median diameter (VMD).

Fig. 2.4 Mastersizer 3000 instrument

2.6.3 Emulsion stability index of Acacia oerfota

Emulsification stability was evaluated by the change in the particle size of emulsion after acceleration test. Emulsion stability index (ESI) was calculated according to PHRC grading system using the equation:

ESI= d0.5as prepared + (d0.53 days@60C - d0.5as prepared) + (d0.57days@60C - d0.5as prepared)

Chapter Three

Results and discussion

3.1 Moisture content

Table 3.1 shows that the moisture content of gum *Acacia oerfota* collected from Senga location ranged between (11.8 - 13.1%) with an average value of 12.45%. These results are the same as the result of the samples collected from Waddalhadad location (11.8-13.4%) with an average value of 12.60% as shown in Table 3.2. These results show higher moisture contents compared to (4.60%) given by Karamallah (1999). These results are conforming to The Sudanese Standards moisture content (not more Than 15%).

3.2 Ash content

Tables 3.1 and 3.2 shows that the ash% of gum *Acacia oerfota* are in the range of (1.12 - 1.40%). The average values is 1.26% which is almost similar to ash content of gum *Acacia oerfota* reported by Anderson (1976) and Karamallah (1999), which was found to be 1.54% and 1.03% respectively.

These results are lower than the results reported by Osman (1993) in the study of *A. senegal* gum collected from different locations of Sudan, he reported an average of 3.6% ash content.

Also the results were less than the values mentioned for *A. senegal* by Karamallah (1998), Omer (2006), Abdelrahman (2008) and Younes (2009), which were reported as 3.77%, 3.27, 3.32 % and 4.89% respectively.

Table 3.1 Physicochemical properties of *Acacia oerfota* gum- Se*n*ga location sample

Number	S. Name	Moisture%	Ash%	pH	S.O.Rotation
1	Sample.1	12.37	1.10	4.88	+80
2	Sample.2	13.65	1.12	5.09	+70
3	Sample.3	11.23	1.16	4.97	+65
4	Sample.4	11.67	1.24	5.15	+60
5	Sample.5	13.46	1.22	4.90	+80
6	Sample.6	12.90	1.34	5.22	+60
7	Sample.7	11.13	1.26	5.09	+80
8	Sample.8	12.5	1.28	5.03	+80
9	Sample.9	12.67	1.22	5.13	+65
10	Sample.10	11.98	1.40	5.26	+65
11	Sample.11	12.79	1.30	5.20	+75
12	Sample.12	11.21	1.30	5.08	+65
13	Sample.13	12.02	1.49	5.26	+80
14	Sample.14	12.25	1.29	5.21	+80
15	Sample.15	11.98	1.26	5.18	+80
16	**Comp.1**	12.35	1.26	4.90	+70
17	**Comp.2**	12.47	1.27	5.15	+65
18	**Comp.3**	12.33	1.24	5.16	+75
19	**Comp.4**	**12.16**	**1.30**	**5.05**	**+70**
Ranges		**11.8 - 13.1**	**1.12 - 1.40**	**4.88-5.26**	**+65** to **+80**

Table 3.2 Physicochemical properties of *Acacia oerfota* gum - *Wadal hadad* aria

Number	S. code	Moisture%	Ash%	pH	S.O. Rotation
1	sample.16	12.8	1.60	4.55	+75
2	sample.17	12.2	1.86	4.94	+65
3	sample.18	12.5	1.56	4.96	+65
4	sample.19	12.7	1.58	4.65	+70
5	sample.20	13.1	1.83	5.02	+70
6	sample.21	12.2	1.43	4.88	+80
7	sample.22	12.6	1.62	4.88	+65
8	sample.23	12.9	1.61	4.94	+80
9	sample.24	13.4	1.27	4.97	+75
10	sample.25	11.9	1.83	4.60	+75
11	sample.26	12.6	1.40	4.90	+75
12	sample.27	11.8	1.53	4.90	+70
13	sample.28	12.4	1.16	5.28	+75
14	sample.29	12.6	1.20	4.87	+70
15	sample.30	13.0	1.65	4.90	+75
16	**Comp.5**	12.2	1.60	4.83	+80
17	**Comp.6**	12.6	1.63	4.90	+75
18	**Comp.7**	12.9	1.31	4.88	+65
19	**Comp.8**	**12.5**	**1.65**	**4.86**	**+70**
Ranges		**11.8-13.4**	**1.18 - 1.31**	**4.55 - 5.28**	**+65to+80**

3.3 pH value

Tables 3.1 and 3.2 show that there is no significant variation in two locations (4.88 - 5.26) for *Senga* location and (4.55 - 5.28) for *Waddalhadad* location, the average value is about 5. Accordingly the gum *Acacia oerfota* has low acidity nature. This results is less than the result reported by Karamallah (1999) which was 3.5. In Comparison to A. *senegal* gum, These results are closed to 4.66 reported by Karamallah *et al.*, (1998) and 4.78 reported by Younes (2009).

3.4 Specific optical rotation

Tables 3.1 and 3.2 shows that the Specific optical rotation of gum *Acacia oerfota* gum is (+65 to +80) with average value 72.5%. This results agree with the result giving by (Karamalla, 1999) which was +64.16. In comparison to A. *senegal*, levorotary reported by Anderson (1977), Osman (1993), Vavdevelde and Fenyo (1985), which were -30º,(-29º to -31º) , and (-29º to -34.4º) respectively. But has a few increase than the results determined corresponding to A. *seyal* by Hassan (2000), Siddig *et al.*, (2005), Omer (2006), Younes (2009), which were (+40º to +62º) , +45º, +49.4º, and +52º respectively.

3.5 Cations composition

Cationic composition studied using Electronic scanning microscope technique. Tables 3.3 and 3.4 showed that Calcium has the highest value among the cations studied in Wadel hadad location, followed by Silicon, potassium, Iron, Titanium, and Strontium ; but Table 3.5 and 3.6 shows that the cations composition of *Senga* location are Calcium, potassium, Silicon, Sulphur, and Iron.

Table 3.3 The cationic composition of A. gum *oerfota* - Wadel hadad location

Element	Result	Unit
Ca	0.287	%
K	0.131	%
Fe	0.010	%
Sr	0.004	%

Table 3.4 The cations composition% to the total amount of cations included in A. gum *oerfota* - Wadel hadad location

Element	Ca	Si	K	Fe	Ti	Sr
Percentage%	58.783	18.670	16.248	3.955	1.285	1.099

Table 3.5 The cationic composition of A. *oerfota* - *Senga* location

Element	Result	Unit
Ca	0.242	%
K	0.145	%
Sr	0.005	%
S	0.002	%
Fe	0.001	%

Table 3.6 The cationic composition% to the total amount of cations included in A. gum *oerfota* - Senga location

Element	Ca	K	Sr	S	Fe
Result	62.633	19.628	12.729	3.549	1.461

3.6 Viscosity

The viscosity of gum *Acacia oerfota* was measured using U-tube Ostwald viscometer. The results is found to be in the range of (3.94 - 10 cm^3g^{-1}) as shown in Fig. 3.1. These results is reflecting its resemblance to *Gummiferae* series, it is characterized by its low viscosity (Anderson *et al*., 1963, 1966). These results is law than the results obtained for *Acacia senegal* (9.7-26.5 cm^3g^{-1}) Al-Assaf *et al*., (2005). Hassan *et al*., (2005) reported (11.9–17.6 cm^3g^{-1}) for A. *seyal*.

Huggins coefficient (k) and (α) was calculated using Mark-Houwink equation, using the relationship between intrinsic viscosity determined by viscometer and molecular mass determined by GPC-MALLS and they were found to be 0.86 and 0.0136 respectively.
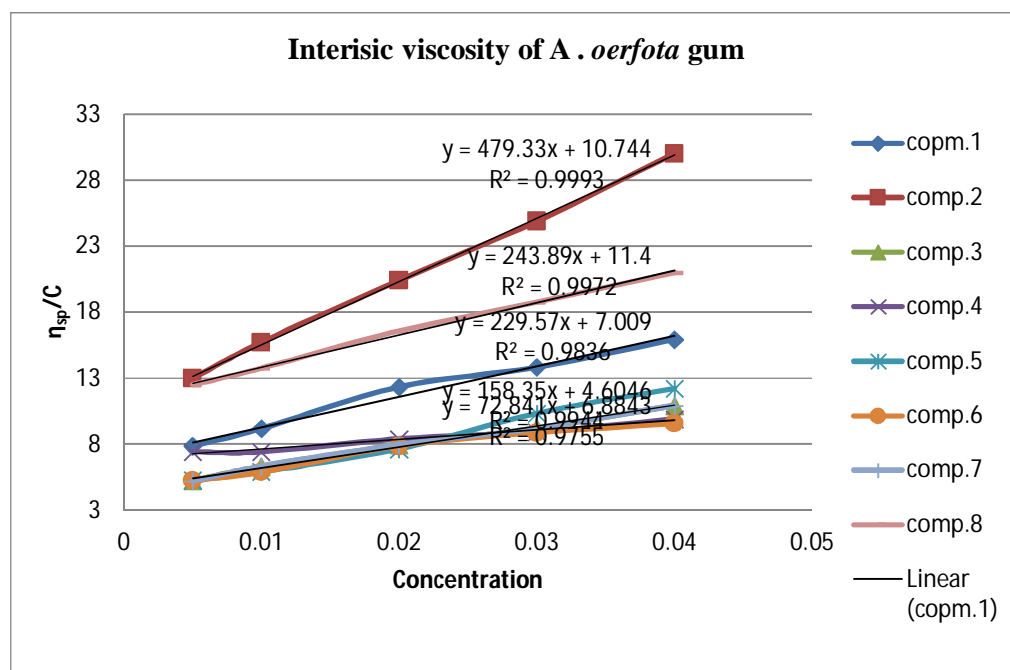
Fig. 3.1 The intrinsic viscosity of six composite sample of A. *oerfota* **gum**

3.7 The Calorific value

Table 3.7 shows that the calorific values of gum *Acacia oerfota* are closely same to the calorific value of the gums, they were about 4 Kcal/g. This calorific value is very low, so the gum *Acacia oerfota* is very suitable to be used as food additives after determination the toxicity value.

Table. 3.7 The calorific values of gum **Acacia oerfota gum**

sample name	sample location	sample weight	bag Cal. value J/g	Cross cal. Value	Net Cal. value j/g	net.Cal.value. cal/g	Cal Value Kcal/g
A.*Oerfota* gum	Senga	0.5189	46463	15805	17024	4067	4.067
A.*Oerfota* gum	Aljazera	0.5066	46463	15596	16829	4020	4.020

3.8 Nitrogen and protein content

The average value of nitrogen and protein content of gum *Acacia oerfota* samples was higher than that values of nitrogen and protein content of *A. senegal* and *seyal.* Table 3.8 shows that the average value of nitrogen and protein content of gum *Acacia oerfota* was found to be 0.53 and 3.28 % respectively. Anderson (1977) reported that nitrogen content of *A. senegal* gum is 0.29% and for *A. seyal* is 0.14%. Osman (1993) reported that nitrogen content for *A. senegal* gum 0.31% and the protein content is 2.4%. Hassan *et al.*, (2005) reported protein content of *A. seyal* had a mean value of 0.96%. Younes (2009) determined nitrogen content for *A. senegal* 0.35% and protein content 2.3%,. Idris *et al.*, (1998) studied the nitrogen content of *A. senegal* from trees of different ages and different locations and they found the range between (0.22- 0.39%), hence protein content ranged between (1.5-2.6%).

Table 3.8 Nitrogen and protein content of A. *oerfota* gum

location	Sample code	Nitrogen content	Protein %
Wadel hadad	**comp.1**	0.49	3.06
	comp.2	0.63	3.94
	comp.3	0.51	3.11
	comp.4	0.49	3.06
Senga	**comp.5**	0.35	2.19
	comp.6	0.77	4.81
	comp.7	0.43	2.71
	comp.8	0.42	2.63

3.9 Equivalent Weight and Total *Uronic Acid*

Table 3.9 shows that the acid equivalent weight and total *uronic* acid % of gum *Acacia oerfota* are found to be in the range of (2941 - 5357) and (3.62 - 6.60) respectively, these results is determined the law acidity of *A.oerfota* in comparison to A. *Senegal* gum, Osman, M.E. reported that the equivalent weight is 1040, and total uronic acid 17% (1993).

Table 3.9 Equivalent weight and Uronic Acid of gum Acacia oerfota

Location	Sample	Acid eq. weight	Uronic acid%
Wadel hadad	**comp.1**	3409	5.69
	comp.2	3571	5.43
	comp.3	3333	5.82
	comp.4	2941	6.60
Senga	**comp.1**	5357	3.62
	comp.2	3846	5.04
	comp.3	3192	6.08
	comp.4	3409	5.69

3.10 Colour Gardner and Tannin content

The colour Gardner of gum *Acacia oerfota* is 0.1 for Senga composite sample and 0.2 for Aljazera composite sample. Table 3.10 shows that the values are increasing with time (24 hours and 48 hours) due to oxidation of polyphenol. Tannin content is calculated according to kjeldahl method, according to table 3.10 the two composite samples have tannin content of about 268.0 and 292.1 mg/g respectively.

Table 3.10 Colour and tannin value of A. *oerfota* and A. *senegal* gum

Sample name	location	After 3 hours					Tannin (ppm)
		off Hue	L*	a*	b*	Gardner	
A. *oerfota*	Senga	0	99.3	-0.02	0.84	0.1	268.0
A. *oerfota*	Wadel hadad	0	98.71	-0.05	1.37	0.2	292.1
		After 24 hours					Tannin (ppm)
Sample name		off Hue	L*	a*	b*	Gardner	(ppm)
A. *oerfota*	Senga	0	99.6	-0.11	0.86	0.1	268.0
A. *oerfota*	Wadel hadad	0	99.14	-0.08	1.46	0.3	292.1
		After 48 hours					Tannin (ppm)
Sample name		off Hue	L*	a*	b*	Gardner	(ppm)
A. *oerfota*	Senga	0	98.04	-0.07	2.09	0.3	268.0
A. *oerfota*	Wadel hadad	0	97.83	-0.17	2.63	0.3	292.1

3.11 Determination of number average molecular weight by Osmotic pressure

The results obtained for gum *Acacia oerfota* is consistent with the observation that *Gummiferae* series possesses usually high molecular weight. Fig. 3.2 shows the values of number average molecular weight (Mn) of gum *Acacia oerfota* obtained by osmotic pressure are 1.68×10^5 and $1.80 \times 10^5 g/mol$ for the two total composite of two locations. These values were lower than the values obtained by gel permeation chromatography $6.23x10^5$ and $9.59x10^5$, the difference in the results is probably due to the differences between the two techniques. The values of number average molecule weight (Mn) of A. *oerfota* gum is not more variable than that of A. *senegal* $2 - 3 \times 10^5$ (Osman, et.all,1993).
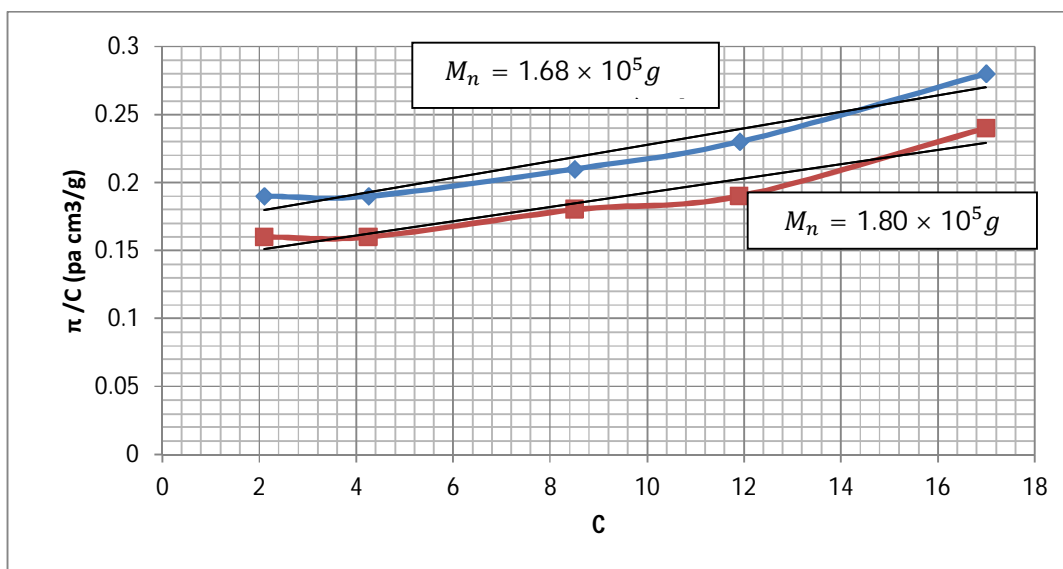
Fig. 3.2 A plot of $\frac{\pi}{C}$ versus C for A. *oerfota gum* at 25 C

3.12 Infrared Spectroscopy (FTIR)

The entire spectrum (FTIR) of gum *Acacia oerfota* identified various functional groups (Figs 3.3). Glucuronic acid has most prominent and very strong proud band covering a wide range of about 3600 - 3000 cm^{-1} for the O - H stretch. At the same time they also show the band in the middle of the spectrum in 1730 cm^{-1} corresponding to the C=O stretching vibration. hydroxyl group of composite sugars and phenol including C - O bond, the stretching vibration appear at 1031 cm^{-1}. The beak appear at 2929 cm^{-1} with bending weak beak around 1400 cm^{-1} is due to aliphatic C - H stretching and pending vibration respectively.
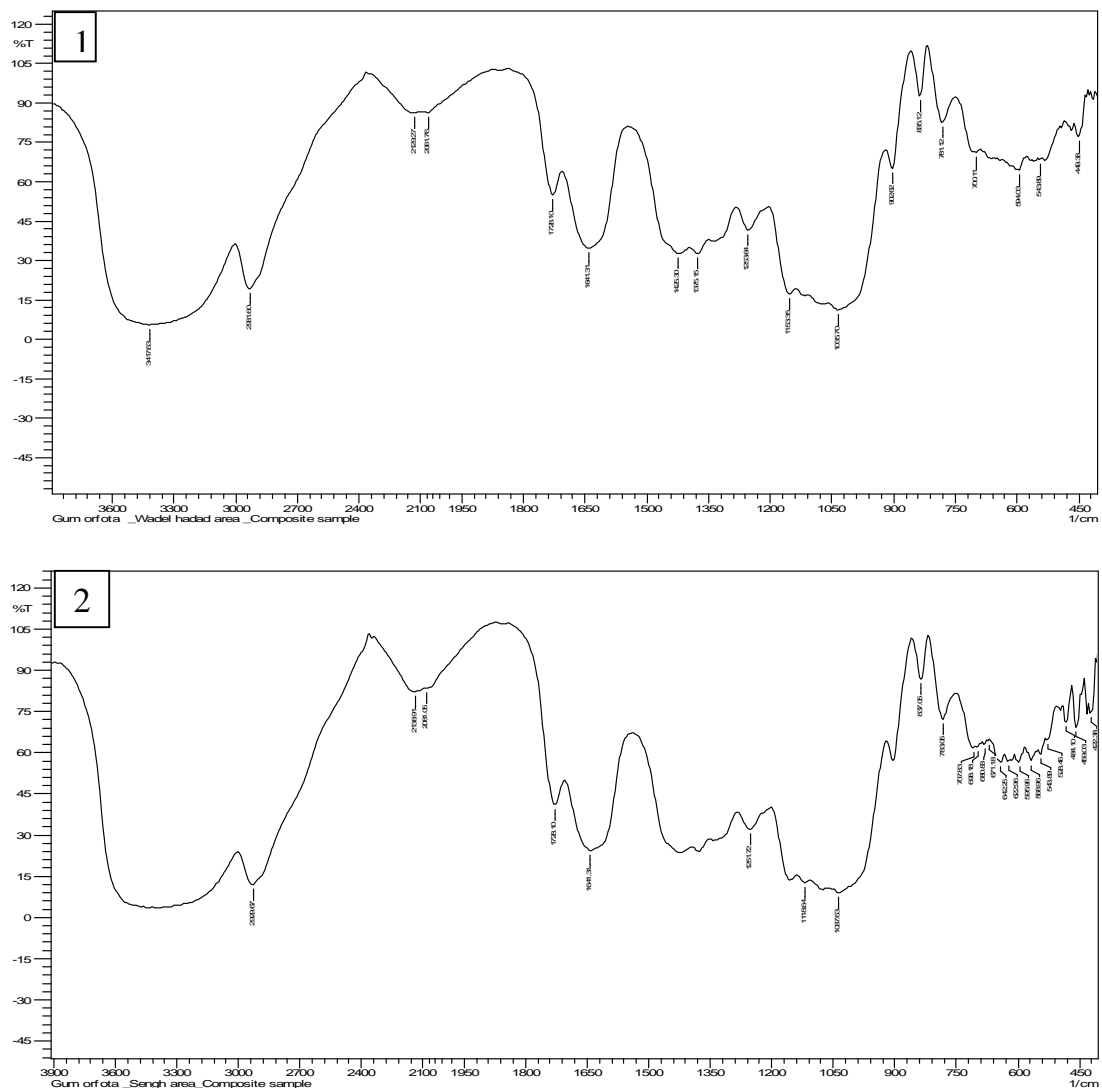
Fig. 3.3 FTIR of composite sample (1) Senga location, (2) Wadel hadad location

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3.13 Rheology of the gum

The Rheological behavior of *acacia oerfota* gum was studied in two deferent concentrations 25% and 50%. According to the Fig. 3.4 , Fig. 3.5 and Fig. 3.6 *Acacia oerfota* gum shows Newtonian behavior (the viscosity is constant with increasing applied shear rate), and the molecules retain initial viscosity on removing the applied stress (Braun, D.B. and Rosen, M.R. 2000).
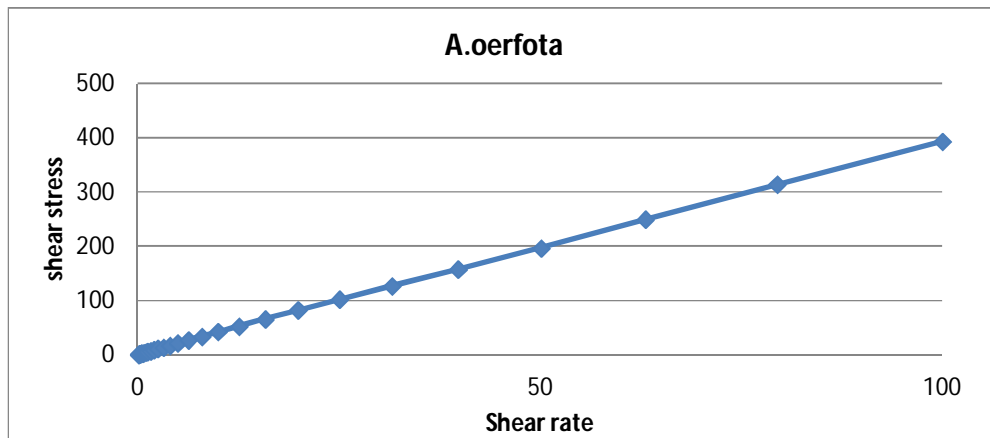
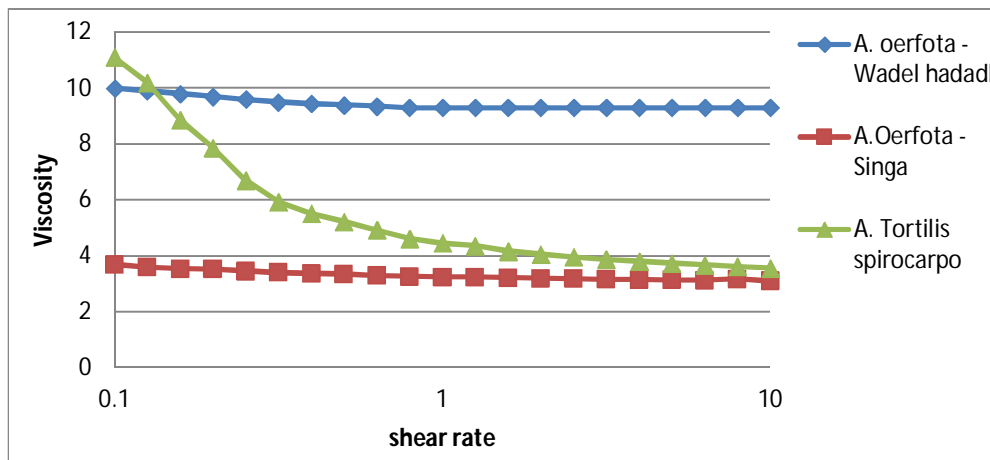**Fig. 3.4 Shear rate versus shear stress of *A. oerfota* gum**

Fig. 3.5 Viscosity profile of Acacia gum emulsions with shear rate - conc. 25%.
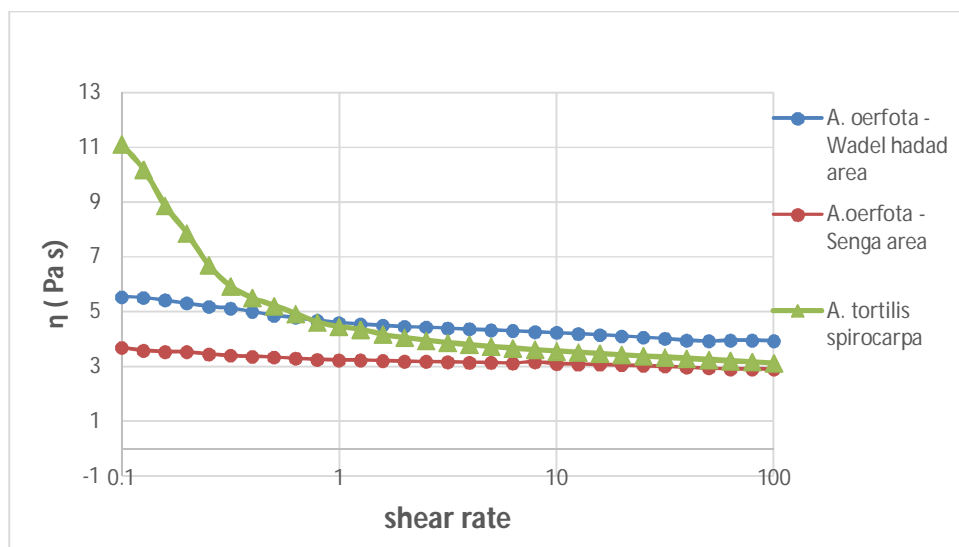
Fig. 3.6 Viscosity profile of Acacia gums emulsions with shear rate - conc. 50%.

3.13.1 The Dynamic Rheology

Fig. 3.7 shows that the loss modulus (G″) of gum *Acacia oerfota* was higher than the storage modulus (G′) and they do not cross, accordingly, gum *Acacia oerfota* is a viscous not, elastic or viscoelastic.

The frequency sweep gives information about gel strength where a high slope of the G' curve indicates low strength and a small slope indicates high strength (Mezger, T. G., 2002). Both gums show similar gel strength.

As G" modulus depends on the frequency but G' modulus depends on the square of the frequency (Barnes HA, Hutton JF, Walters K. 2001). G' becomes more important at higher frequencies. In comparison to *A. Tortilis* G" is greater than G', then A. *Tortilis* have viscose properties at lower frequency. With increasing frequency systematically throughout a wide range, the solution of *A. Tortilis* is changes from viscose to elastic properties (Mezger, T. G., 2002). *A. Tortilis* forms strong gels above critical frequency (0.5 HZ).
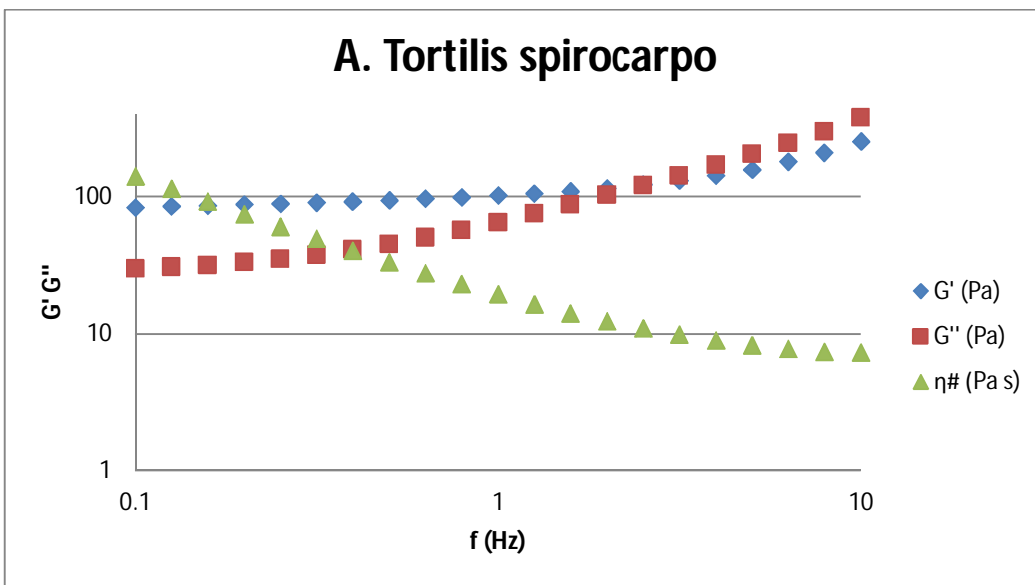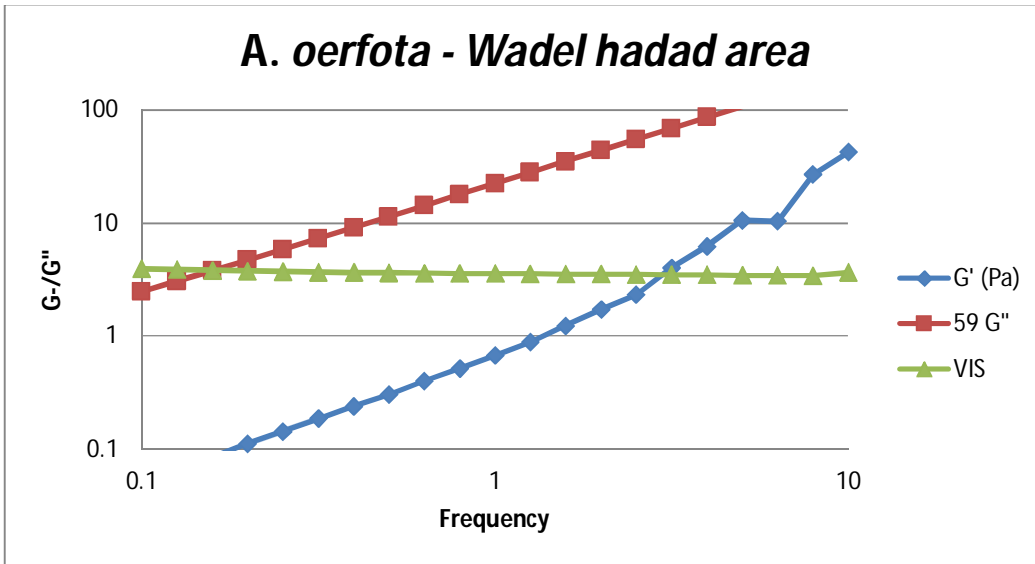
A. *oerfota - Wadel hadad area*

Legend:
- G' (Pa)
- 59 G''
- VIS

A. Tortilis spirocarpo

Legend:
- G' (Pa)
- G'' (Pa)
- η# (Pa s)

Fig. 3.7 The effects of frequency on G' and G''

3.14 Gel permeation chromatography (GPC MALLS)

GPC-MALLS with on-line monitoring using, RI, LS and UV absorbance detectors.

3.14.1 GPC MALLS - RI

Fig. 3.8 shows RI profiles of A. *oerfota* and A. *senegal* gums. The profile is composed of three peaks AGP, AG and GP. There is quite different in the first peak of AGP. The mass % of AGP of the two A. *oerfota* gums are almost similar, but very low in comparison with the AGP of A. *senegal* gum.

3.14.2 GPC MALLS - LS

Fig. 3.9 shows that light scattering profiles for AGP and AG of the two *A. oerfota* and *A. senegal* gums. (GP is not detected due to small M_w). The first peak reflects the significant difference in molecular weights of Arabinoglactan protein components in *A. Oerfota* and *A. senegal* gums. The molecule weight of the Arabinoglactan protein in *A. oerfota* is very low in Comparison to that of *A. senegal* gum. However, The AG+GP molecular weight of *A. oerfota* is higher than that of *A. senegal* gum.

3.14.3 GPC MALLS - UV

Fig 3.10 shows the UV, GPC profiles for Acacia *oerfota* and *A. senegal* gums. They, clearly, indicate the significant difference in protein content. The AGP signal of *Acacia oerfota* gum is very small in comparison to that of *Acacia senegal* gum. The AG and GP signals are almost identical, indicating similar protein composition.
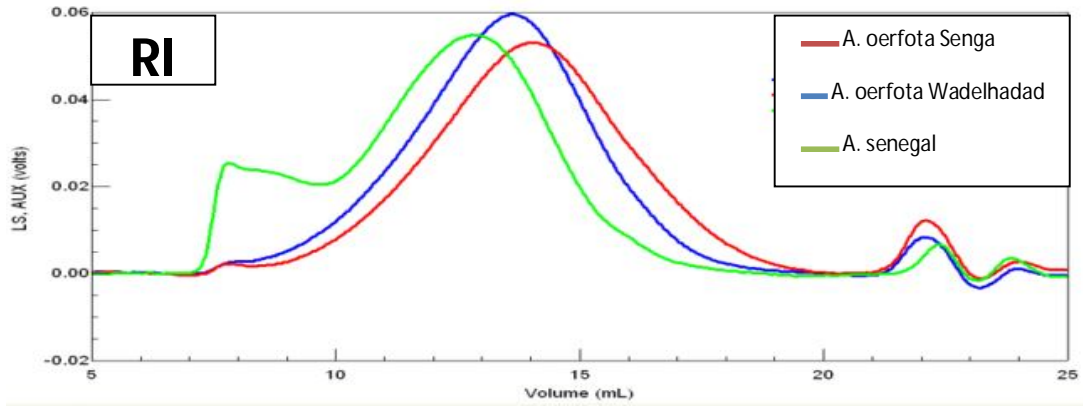
Fig. 3.8   The RI of two Composite samples of A. *oerfota* and A. Senegal)
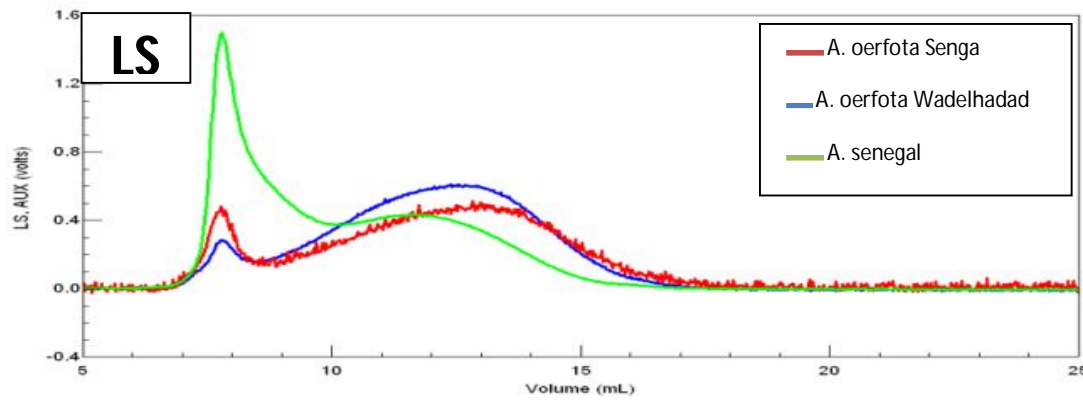

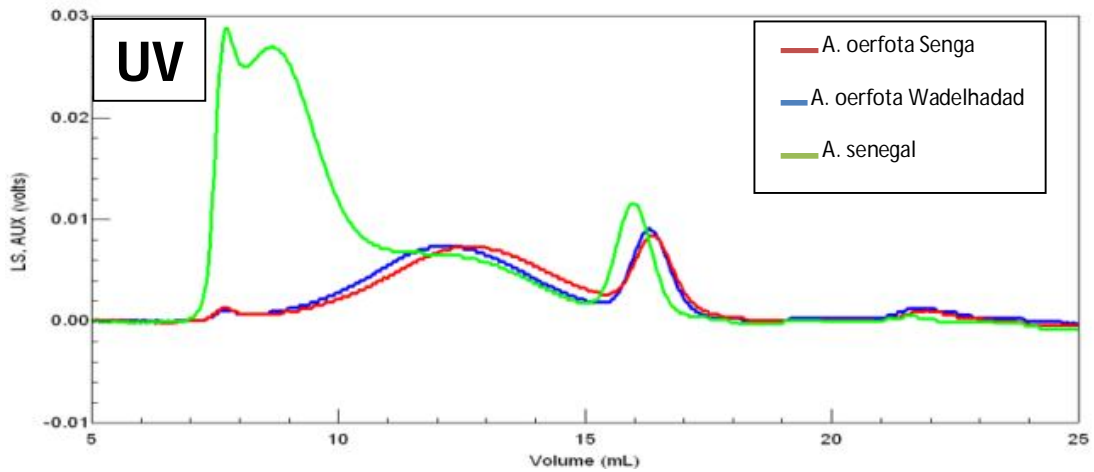**Fig. 3.9 The LS of two Composite samples of A. *oerfota* and A. Senegal**

Fig. 3.10 The UV of two Composite samples of A. *oerfota* and A. Senegal

Table 3.11 The GPC-MALLS result for A. oerfota and A. senegal gum

function/sample	A.oerfota.1	A.oerfota.2	A.senegal
Mw whole gum ($\times 10^5$)	6.23	9.59	5.95
% Mass recovery	108.685	105.78	113.97
Rg (whole gum)/nm	178	178	50.6 (6%)
Mw AGP	4.77	14.19	1.68
% mass (AGP)	1.11	0.84	17.73
Rg-AGP	50	187	38. (3%)
Mw (AG+GP) ($\times 10^5$)	5.76	8.24	3.59
% mass (AG+GP)	98.89	99.16	82.27

3.15 The Emulsification Properties of *Acacia oerfota* gum

Good emulsions are characterized by small particle diameter size between 0.1 and 1 micron and a narrow band. The emulsification properties of A. *oerfota* gum were studied by determination of the emulsion particle size at zero time and after incubation for 3 and 7 days at 60 C^0.

Table 3.12 shows that the location have no significant effect in the emulsification properties of A. *oerfota* gum. The surface weighted mean D(3,2) and volume weighted mean D(4,3) was increasing and the span% was decreasing with time.

D(4,3) is more sensitive to the existence of large particles in an emulsion compared to D(3,2). Thus, D(4,3) more sensitive to the phenomena of flocculation. This might explain the higher value of D(4,3) compared to D(3,2) since all emulsions showed large droplets. The gum is designed as grade 3 According to emulsion stability index value that taken as a parameter to evaluated the grade of the gum sample.

Table 3.12 The Emulsification Properties of *A. oerfota* **gum**

Senga location				Wadel hadad location		
Character	As prepared	After 3 days	After 7 days	As prepared	After 3 days	After 7 days
D(3,2)	0.674	1.81	1.99	0.364	2.08	1.79
D(4,3)	1.26	4.43	4.34	1.56	5.42	4.46
span%	2.32	1.95	1.9	1.98	1.74	1.68
Dx(10)	0.40	0.69	0.74	0.166	0.63	0.58
Dx(20)	0.49	0.96	1.16	0.238	2.95	2.17
Dx(50)	0.72	4.26	4.08	0.477	3.23	4.42
Dx(80)	1.1	7.3	6.91	3.51	8.1	6.63
Dx(90)	2.1	8.98	8.49	5.4	9.71	7.98
Grade	3	3	3	3	3	3

Fig. 3.11 shows more variable particles size distributed in wide range (0.1 - 10) micron and most particle diameter size is large, this indicate instability of *Acacia oerfota* gum emulsion (Billmeyer, F. W. 1971). The emulsions exhibited a typical bimodal droplet size distribution with a pronounced shoulder reflecting a two groups of the droplet with the small and largest diameter. The small one in the range of good emulsion particle size, but it decreasing with time according to flocculation and coalescence process. The pig droplet size and low viscosity (3.4 - 11.4 cm3g^{-1}) and pig rate of gyration of A. *oerfota* gum supported instability of the emulsions.
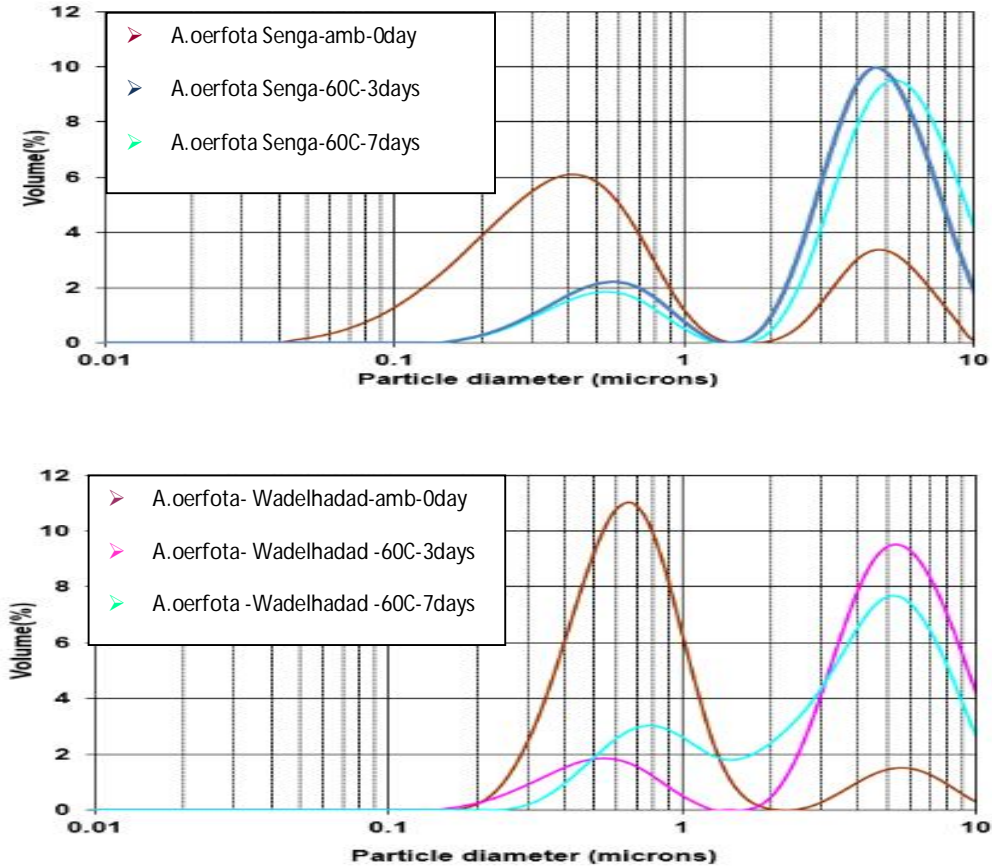
Fig. 3.11 The emulation particle size profile of *A. oerfota* from two locations

Figs. 3.12 show high value of polydispersipility index (span %) for the fresh emulsion and after incubation for 3 and 7 days at 60ºC respectively. Two locations indicating a bad uniformity of the droplet size. These values reflecting the instability of the emulsions by lowering the amount of protein that associated with surface of the emulsion droplet.
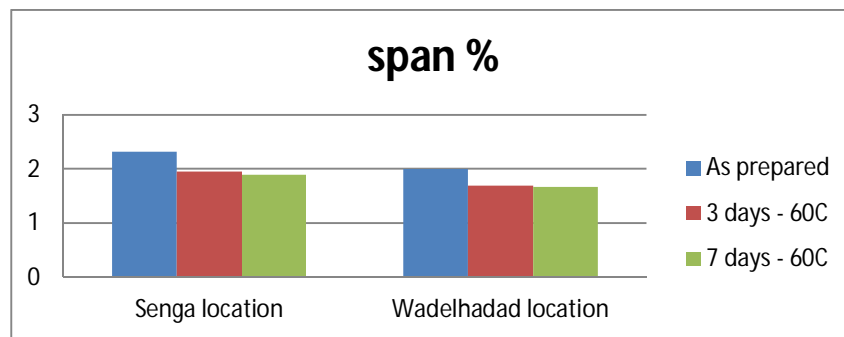

Fig. 3.12 The span% of A. *oerfota* gum from two location

Figs. 3.13 Show specific surface area (m^2/g) of cumulative droplet distributions of (D.5, D.9, >1 microns, and >2 microns). The results clearly showed that extreme changing was found in emulsions during the incubation for 3 and 7 days at 60°C. Also the instability of the emulsion enhanced by decreasing of D(0.5) area to less than 6%, and increasing the droplet particles size with the diameter of more than one microns to more than 80%.
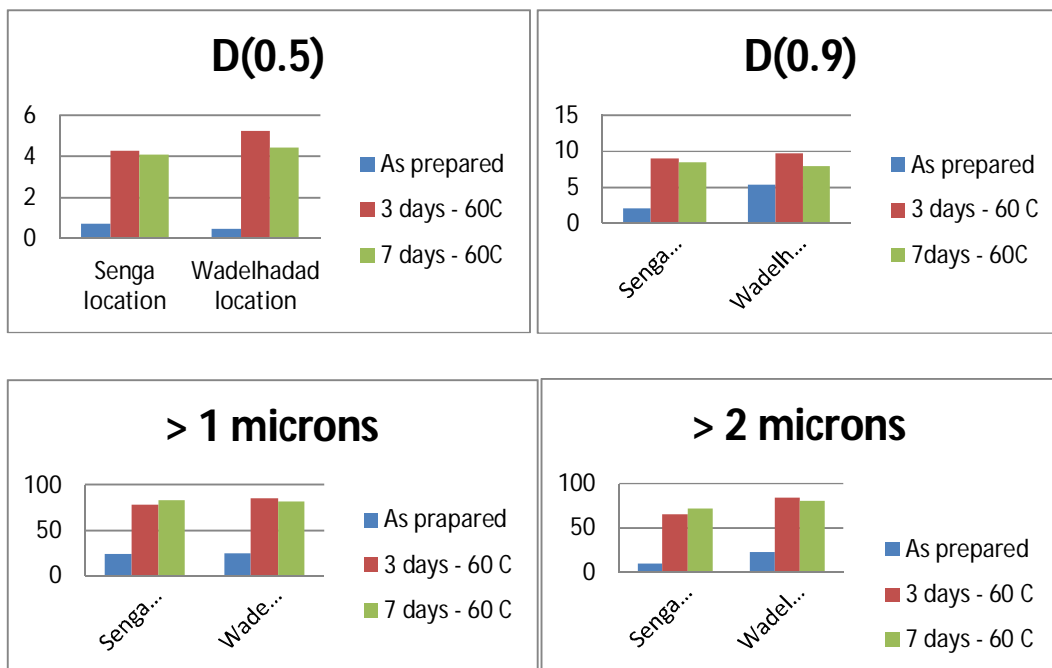

Fig. 3.13 The Volume Weighted Mean Diameter of A. *oerfota* gum Emulsion from two Location.

Conclusions

- Gum *Acacia oerfota* shows physicochemical properties reflecting its resemblance to Gummiferae series.

- The rheology of gum *Acacia oerfota* solution shows that the gum is form a Newtonian fluid.

- The loss modulus of A. *oerfota* gum is higher than the storage modulus.

- The molecular weight of *A. oerfota* gum ranges between (6.23×10^5 and 9.59×10^5 g/mole) with the same radius of gyration. The *Arabinoglactan* protein has only 1% mass and low molecular weight in Comparison to *A. senegal.*

- Gum *Acacia oerfota* forms poor unstable emulsion.

further work

❖ Toxicology behavior of the gum needs to be investigated.

❖ fractionation of the gum and investigation of the characteristics of the fractions are required.

❖ To improve stability of the emulsion by incorporation of protein or blending with other gums of high protein content.

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