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Emulsifying and Rheological Properties of *Acacia tortilis* var. *spirocarpa* Gum from Sudan

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Abstract

This work aims to study the emulsification and rheological properties of *Acacia tortilis* var *spirocarpa* gum. A composite sample of the gum was prepared from samples collected from Aboshmal forest Sharq Elneel, Khartoum state. Emulsions, of two samples, concentrations (5 and 10% w/w) and dioctanoic decanoic glycerol (Octanoic Decanoic triglyceride oil) (ODO), were prepared, by homogenization at 75MPa and stored for 3 and 7 days at 60**C**. The emulsifying stability was determined by the change in droplet size using a Mastersizer 3000 laser diffraction analyzer. The Emulsification study showed that *A. tortilis* var. *spirocarpa* gum is grade 1 emulsifier. The Flow behaviour and dynamic properties for two gum samples, at 25 and 50% w/w concentrations, were determined by applying a shear rate of 0.1-100$S^{-1.}$ Rheological studies of *A.tortilis* var..*spirocarpa* showed that the gum had formed a Newtonian fluid at high shear rate and high concentration and the result of oscillatory test of the gum solution revealed a solid-like more than a liquid-like behaviour.

Keywords: A.tortilis var.spirocarpa Gum, Emulsification,Octanoic Decanoic triglyceride oil , Rheological properties

المستخلص:

يهدف هذا العمل إلى دراسة خصائص الاستحلاب والانسيابية في صمغ السمر. تم تحضير عينة مركبة من الصمغ من عينات مأخوذة من غابة أبوشمال شرق النيل بولاية الخرطوم. . تم تحضير مستحلبات من عينتين بتركيزات (5 و 10٪ وزن / وزن) وزيت ثنائي أوكتانويك ديكينويك الجلسرول(اوكتانويك ديكانويك ثلاثي الجلسريد) (ODO) ، عن طريق التجانس عند 75 ميجا باسكال وتخزينها لمدة 3 و 7 أيام عند 60 درجة مئوية. تم تحديد استقرار الاستحلاب من خلال التغيير في حجم القطرة باستخدام محلل حيود الليزر اظهرت دراسة الاستحلاب ان صمغ السمر يختلف عن بقية الاصماغ الاخرى فهو مستحلب من الدرجة الأولى. وتم تحديد سلوك التدفق والخصائص الديناميكية لعينتين من الصمغ ، بتركيزات(25 و 50٪ وزن / وزن) ، من خلال تطبيق معدل القص 0.1-100ث واظهرت الدراسات الريولوجية لصمغ السمر انه يشكل سائلًا نيوتونيًا بمعدل قص عالٍ عند تركيز عالٍ ، وأظهرت نتيجة الاختبار التذبذبي لمحلول الصمغ انه يسلك سلوك ماده شبيه بالجوامد أكثر من سلوك ماده شبيه بالسوائل.

الكلمات المفتاحية: صمغ السمر. ، الاستحلاب ,اوكتانويك ديكانويك ثلاثي الجلسريد ، الخواص الريولوجية.

Introduction

Emulsion is a dispersion of two immiscible liquids such as oil-in-water (O/W) (Dickinson,1992), thermodynamically unstable system , separates into two phases of a period of time , is stabilized by improving its kinetic stability (Bergenstahl,B.A.1997) .Gums are biopolymers (FAO,1999) , water soluble up to 50% concentrations, have low viscosity (Dauqan *etal*.2013). used as stabilizers, thickeners, emulsifiers and encapsulating agents in confectionery ,bakery, dairy products beverage (Verbeken *et al*.2003, Sanchez *etal*.2008).

which are composed of carbohydrates and proteins, the carbohydrate moiety is hydrophilic while the protein moiety is hydrophobic (Dickinson, 1992) , which are composed of three main fractions, a highly branched polysaccharide fraction arabinogalactan AG (90%), a small fraction with high molecular weight, an arabinogalactan protein AGP (10%) and glycoprotein GP (1%) (Randall *etal*.1989, Osman *etal*., 1995, Islam *etal*.,1997, Renard *etal*,2006) The great flexibility of AGP structure allows molecules to be easily deformed at oil/water interfaces (Sanchez *etal*.2002). The emulsion stabilization, is mainly attributed to the charged and hydrated carbohydrate portion, (Chanamai and McClements,2001), the hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and steric repulsion (Dickinson, 2003, Lelon *etal*, 2010). The increase of AGP percentage in emulsion is helped to achieve more uniform homogeneity of oil droplet population (Hamouda,2017).

Emulsifying properties of the gum depend on the total protein content and the distribution of the protein/peptide between the low and high molecular weight fractions and on the molecular accessibility of the protein/peptide for adsorption (Randall *etal*.1989, Dickinson *etal*.1991). Removal of the protein by treatment with proteolytic enzyme reduces the emulsification properties (Randall *etal*. 1988). Previous studies have shown the stability of oil in water emulsions (O/W) depends on both the type and concentration of ingredients contained into emulsion as well as processing and storage conditions (Mc Clements,1999)., this is in agreement with the study done by (Sabah Elkhair,2008) emulsion stability affects by the type of oil , stirring time and also gum grade but the study is,disagreed in factors like concentration and temperature that does not influence emulsion stability. Minerals decrease emulsion stability due to an electrostatic screening effect, pH also affects stability, when it is 2.5 stablility decreases and when it is (4.5-5.5) stability increases. (Dickinson,*etal* 1991).

The stability Index for 5% concentration of *A. senegal* var *senegal* and *A.mellefera* gum dispersion in D-lemonene oil exceed 90%, while *A. seyal* var *seyal* and *A. tortilis* var *raddiana* more than 80%, the high concentration of gum reduced the creaming and increase the emulsion stability.(Daoub,2016).The volume median diameter (VMD) droplet size for *A. senegal var senegal* 30% wt gum solution in medium chain triglyceride oil,, is greater than 1 micron (Hamouda, 2017).

The rheological properties of acacia gums play a significant role in the food industry as they govern the product development, design and evaluation of the process and also affect flow behaviour of the products (Bolmstedt, 2000). Rheological properties of dilute *Acacia gum* solution have not been considered in great details since the Newtonian character is assumed a priori. At concentrations between 10 - 25% acacia gum dispersion exhibits more pronounced shear-thinning behaviour than dispersions with concentrations in the range 30-50%. (Sanchez *etal.* 2002). Non-Newtonian behaviour of gum solutions is induced by the presence of molecular associations (Li *etal*.2011).The occurance of AGP micelles endows the gum Arabic solution with Non-Newtonian and shear thinning behavior (Li *etal,*2011) .The flow of gum solutions shows nearly Newtonian flow for 50% concentration for *A senegal* var *senegal* and 50% for *A seyal* var *seyal* . (Daoub,2016). The apparent viscosity values increase when gum concentration rises.for gum exudates from *A.senegal* (Mothè *etal*,1999) .

Acacia nilotica gum solutions exhibit a high viscosity at low shear rates and a Newtonian region at high shear rate similar to that found for *A.senegal*.(Elhag,S.A.,2018).

Viscoelastic properties of Arabic gum dispersions reveals a predominant liquid-like behavior. Mechanical spectra obtained at 6 wt % AG concentration by oscillatory testing reveals that the viscous-like modulus is high than the elastic-like modulus, after 120 min rest the dynamic mechanical spectra shows a typical gel-like behavior.(Sanchez,2002).The effect of temperature in the dynamic rheological behaviour of *A.senegal* var *senegal* and *A. seyal* var *seyal* , at temperature less than or equal 60C reflects a viscous behaviour and at temperature 70C the behaviour becomes elastic at low frequency region for *A.seyal* var *seyal* while *A senegal* var *senegal* has completely different behaviour.(Elhag,2018)

Acacia tortilis var. *spirocarpa* often called umbrella thorn spreads in seasonally dry areas of Africa and Middle east from Senegal to Somalia. In Sudan, Kenya and Tanzania, middle east, Asia (Palestine, Jordan and southern Arabia to Iran) *A. tortilis* forssk Hayne (subfamily *mimosoidae*, family *leguminosae*) has spread in middle and east , arid and semi arid areas of Sudan, includes subspecies *A. tortilis* var.*spirocarpa* , *A. tortilis* var. *raddiana and A. tortilis var tortilis* (Brenan,1983). The physiochemical properties show that *A.tortilis* var *spirocarpa* gum contains 10.8% moisture, 42.6% arabinose, 14.8% galactose, 3.8% rhamnose, 8.3% uronic acid, 11.2% protein. Minerals in ppm are 8222Ca, 2203K, 1578Mg, and 64Na. A molecular weight of 4.6×10^5. Radius of gyration 35nm. (Mohamed *etal.* 2021).

The objective of this work is to study the functional properties i.e. emulsification and rheology of a promising gum from *A.tortilis* var. *spirocarpa* to cover the lack of knowledge in this area.

2. Materials and Methods:

2.1 Materials:

Authentic sample of naturally exuded gum samples were collected during (April – Jun, 2014) from Aboshmal forest – Sharq Elneel, Khartoum State. The collected gum was relatively pure. Impurities like wood, sand and tree leaves were carefully removed. The samples were air dried, ground using a mortar and pestle then kept in closed plastic containers.

2.2. Emulsion Preparation:

20% w/w gum solution was prepared. The sample was dissolved using a roller mixer (SRT9 - Stuard Scientific, UK) and centrifuged using (Megafuge 1.0R), Heraeus SEPATECH) for 10 min. at 2500 rpm to remove insoluble particles and filtered using 100 µm mesh.

0.52 ml of (10% w/v) sodium benzoate solution and 0.48 ml of 10% (w/v) citric acid solution were added. Distilled water was added to make a 20g total weight of gum solution, then 20g of ODO oil (10%) were added to the prepared gum solution, to give a total weight of 40g and final gum concentration of 10%. The mixed solution was homogenized for 3 min. using a POLYTRON (PT2100, KINEMAX TICA AC) homogenizer at 2200 rpm. Impeller (PTDA 21.9 mm tip diameter) was used as dispersing tool. The pre-emulsified mixture was homogenized using a high – pressure Nano Vater (NV30-FA, MITSUBISHI GDT 1000), passed twice at 75 MPa. The final emulsion was kept in closed glass universals.

The prepared emulsion sample was placed at 60**C** in the vacuum oven (GALLENKAMP-OVA031XX1.5) for 3 and 7 days. The droplet size distribution of the emulsions was measured using Mastersizer3000, a Laser diffraction particle size analyzer (Malvern Instruments).

2.3. Rheological Properties:

50 % w/w gum solutions (on dry weight basis) containing (0.005% w/v) NaN_3 as a preservative were prepared. The solutions agitated on tube roller mixer (SRT9. Stuart Scientific, UK) overnight to ensure complete dissolution. The solution was centrifuged for 10 minutes at 3000 rpm using (Megafuge 1.0R, Heraeus SEPATECH, Germany) centrifuge. 25% (w/v) solution was prepared from stock solution. Rheological measurements were carried out using KINEXUS pro (Malvern Instruments) fitted with cone and plate geometry with a cone diameter of 40 mm and an angle of 2°. Steady shear viscosity curve was measured for gum solutions 25% and 50% w/v both upon shear rate ramp-up (from 0.01 to $10000S^{-1}$), and subsequent shear rate ramp-down (from 10000 back to $0.01S^{-1}$). Dynamic rheological measurements were performed in the frequency range of (0.1-10 Hz) to determine the elastic modulus (G`), viscous modulus (G``) and dynamic viscosity. The linear viscoelastic region was assessed at (1 Hz). The temperature of the samples was controlled within 0.1**C** using a Petlier element. The rheometer control and data processing were done by computer software (Rheology Advantage Data Analysis program).

3. Results and Discussion:

3.1 Emulsion properties of *Acacia tortilis* var. *spirocarpa Gum.*

Table (1): Laser Diffraction measurement of emulsion characteristics of *A.tortilis* var. *spirocarpa* Gum.

Time/Parameter	Concentration	Fresh emulsion	3 days @ 60C	7 days @ 60C
Span	5%	2.557	4.711	4.218
	10%	2.876	3.539	3.274
Specific surface area m^2/kg	5%	68.270	89.860	88.570
	10%	68.850	61.710	60.978
$d_{(4,3)}$	5%	0.480	0.515	0.459
	10%	0.243	0.475	0..309
$d_{(0.1)}$	5%	0.0295	0.0238	0.0241
	10%	0.0382	0.0416	0.0426
$d_{(0.5)}$ VMD	5%	0.376	0.247	0.266
	10%	0.164	0.196	0.196
$d_{(0.9)}$	5%	0.991	1.190	1.140
	10%	0.510	0.735	0.683

Table (1) showed a measured parameters for two samples of gum concentrations (5%, 10% w/w), for each three samples (fresh, stored for 3 and 7 days at 60**C**), using laser diffraction Malven Mastersizer 3000. Specific surface areas were ranged from 68.3 – 88.6 m^2 /kg for 5% and from 68.9 – 60.9 m^2/kg for 10% concentration. On doubling the concentration, it was observed that the change was decreased. The interfacial activity is attributed to the presence of the proteinaceous moiety and the specific high molar mass of AGP concentration. (Randall *etal* ,1989; Alassaf *etal*., 2006), the Higher the protein content, The higher is surface activity (Mahfoudhi *etal.*2014). Droplet size distribution is expressed by polydispersity which in a good uniformity varied between 1.2 to 1.1% for 5% and from 2.3 - 3.5% for 10% concentration. Acceleration and storage, conjoined an increase in droplet size distribution.

A gradual smaller change was observed from the fresh sample to that stored for 7 days at 60**C**, which bounced back between 3days and 7 days at 60C.The mean droplet diameter was expressed as the volume mean diameter $D_{4,3}$. the variability of $D_{4,3}$ was 0.48-0.45 for 5% and 0.24-0.30 for 10% concentration despite few large droplet size diameters were formed, stabilization of emulsion was observed. An increase of the, relatively, high Mw protein rich AGP induced a decrease of $D_{4,3}$. Doubling concentration of AGP produce excellent results and multiplying furthermore the AGP forms stable emulsion of larger droplet sizes (Han *et al*. 2019). The stability of emulsion, obviously, decreases with the storage duration but destabilization was impaired as the AGP protein concentration increases (Aphibanthamakit *et al*, 2020). There is a combined effect of high Mw protein rich AGP and total gum concentration on droplet diameter (Aphibanthamakit *etal*, 2020). The AGP with Mw higher than 10^6 g.mol^{-1} is protein poor and AGP of Mw lower than 10^6 g.mol^{-1} is protein rich , simultaneously, affect the stability of emulsion. (Randall *etal*.1989; Renard *et al*. 2006) Well distribution of protein between protein rich AGP and protein poor AGP affect the proper stability of emulsion.

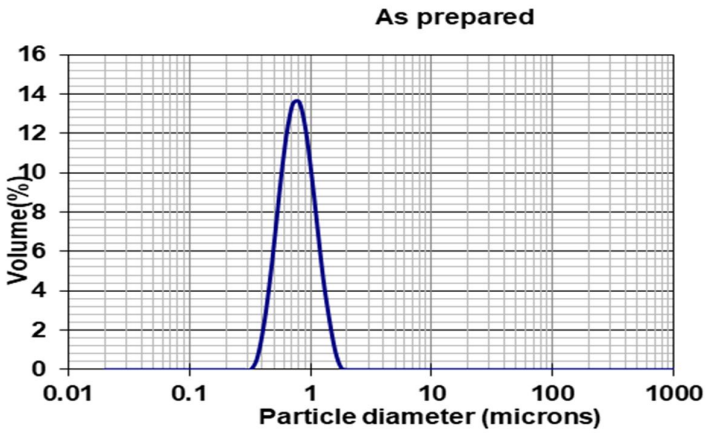

Fig1 Particle-size distribution of *A. tortilis* var. *spirocarpa* Prepared Emulsion (composite sample)

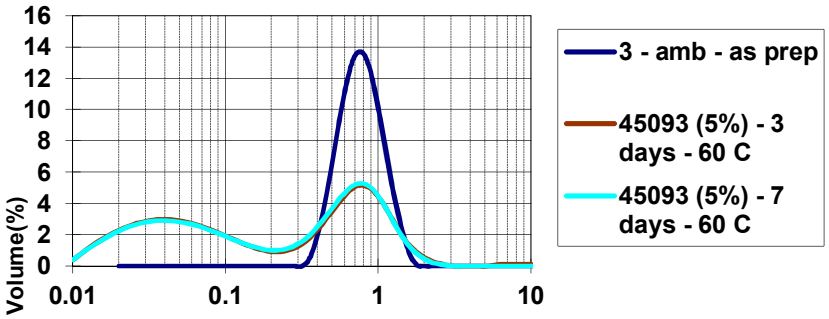

Fig. (2) Particle size distribution of *A. tortilis* var. *spirocarpa* Emulsion and Stored for 3 and 7 days 5% Conc. (composite sample)

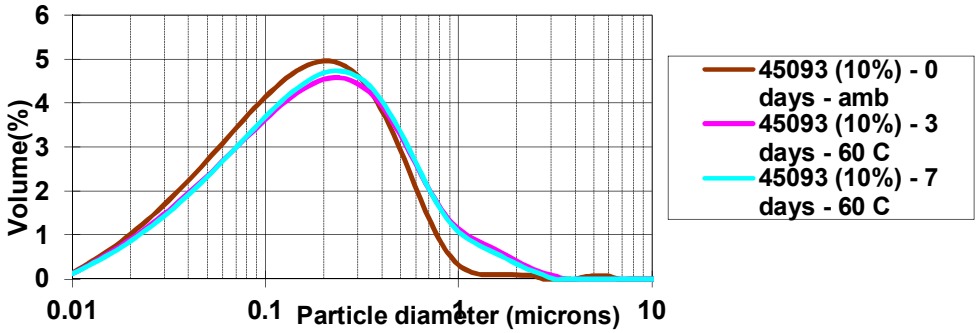

Fig.(3) Particle size distribution of *A. tortilis* var. *spirocarpa* Prepared Emulsion & Stored at 3-7 days 10%Conc. (composite sample).

Figs 1, 2, and 3 showed particles size distributing in three decades. The cumulative droplet distribution d0.1, d0.5, and d0.9 where 0.1, 0.5, and 0.9 represent the droplet size diameter in μ below which the particle size falls. The VMD is the volume median diameter d0.5, for both concentrations the measurement showed a dubitative change in VMD from fresh sample to that be stored at 7 days at 60ºC for 5% concentration , and an increase in VMD for 10% concentration was observed. The majority of particles had sizes ranging between 0.3 to 0.7μ. On acceleration small particle tended to coalesce with others forming particles with size less than 0.7μ. Particles smaller than 1 μ have difficulty overcoming Van Der Waals forces and separates. Emulsion Stability Index ESI was calculated according to Phillips Hydrocolloid Research Center (PHRC) grading system using the equation:

$ESI = d_{0.5(\text{as prepared})} + (d_{0.5(3 \text{ days } @ 60°C)} - d_{0.5(\text{as prepared})}) + (d_{0.5(7 \text{ days } @ 60°C)} - d_{0.5(\text{as prepared})})$, where $d_{0.5}$is the valume median diameter.

Emulsifiers that show a particle size change ≤ 0.7μ is classified as grade 1. ,(very good emulsifier), emusifiers that show a change between 0.7 - 0.85μ classified as grade 2 (good emulsifiers). A particle size changes > 0.85μ are allocated grade 3 (poor emulsifier).

A.tortilis var. *spirocarpa gum* is accordingly classified as a very good emulsifier. The result obtained from *A.tortilis* var *spirocarpa* in a good agreement with the other varients *A.tortilis* var *raddiana* (Abdelrahman,2011) and *A.tortilis* var *tortilis* (Alnour,2014) at the same experimental conditions (oil type, concentrations, processing , temperature) , there was insignificant difference in the parameters span, specific surface areas , $d_{0.5}$ also had semi similar ranges and the ESI for the three varients were less than 1 μ, all of them were considered as grade 1 a very good emulsifiers.

3.2. Rheological Properties:

Fig. 4 represents concentration (25% w/w) The profile started at viscosity 5Pa.s at shear rate 0.1 1/s and then the viscosity decreased with increasing shear rate. The apparent viscosity attained a first Newtonian Plateau at 0.6 1/s shear rate and viscosity 1.7 Pa.s. The curve showed Non-Newtonian fluid, the viscosity depends on shear and shear-thinning dominates.The steady shear flow for *A.tortilis* var *raddiana* (Abdelrahman ,2011) and *A.tortilis* var *tortilis* (Alnour ,2014) at the same condition also show very clear thinning behavior.

Fig. 5. At concentration (50% w/w) The profile started with high viscosity 11 Pa.s at shear rate of 0.1 1/s. The curve started with a nearly Newtonian fluid until the viscosity reached 4 Pa.s with shear rate 1.5 1/s, then the ratio between shear stress and shear strain be constant until shear rate was (80 1/s) the solution behaved as a Newtonian fluid. The graph has shown the clear – zero – viscosity plateau around (0.63 1/s), shear rate and viscosity around (4.9-4.6 Pa.s).

The decrease in viscosity with increasing shear rate is a behaviour of typical of polysaccharide systems which is attributed to the disentanglement of macromolecular chains under shear (Mothè and Rao, 1999). (25% w/w) gum solution, at low flow rates, molecules were long and this has effectively large cross sections due to their tumbling in solution, the author attributed this to existence of AGP micelles which have high radius of gyration value, 53nm (Mohamed *etal*.2021) less compact, highly branched and oriented at different directions (AGP oblate ellipsoid particle with a central intricate network Sanchez *etal*.2008; Renard *etal*., 2012), it resisted the flow of solution but on increasing shear rate, at zero- shear- rate the aggregated structures broke down to primary particles and elongated particles aligning with flow giving much smaller effective cross-sections and hence much lower viscosity due to degradation of AGP to small fragments which supposed to be AG and GP micelles (GP = 9nm, ring-like shapes, Renard *etal*.2014) , more compact molecules, their orientation were not affected the flow of solution . The viscosity decreased with increasing shear strain rate. Therefore, with shear thinning behaviour.the viscosity was Non Newtonian.

At (50%w/w) concentration: Increasing the gum concentration i.e AGP micelles was high concentrated , increased the apparent viscosity and resulted in increasing restriction of molecular movement, Furthermore, the applied shear rate was not directly proportional to the amount of AGP micelles , so increasing of shear rate had less effect on AGP and the viscosity exhibited a Newtonian behaviour

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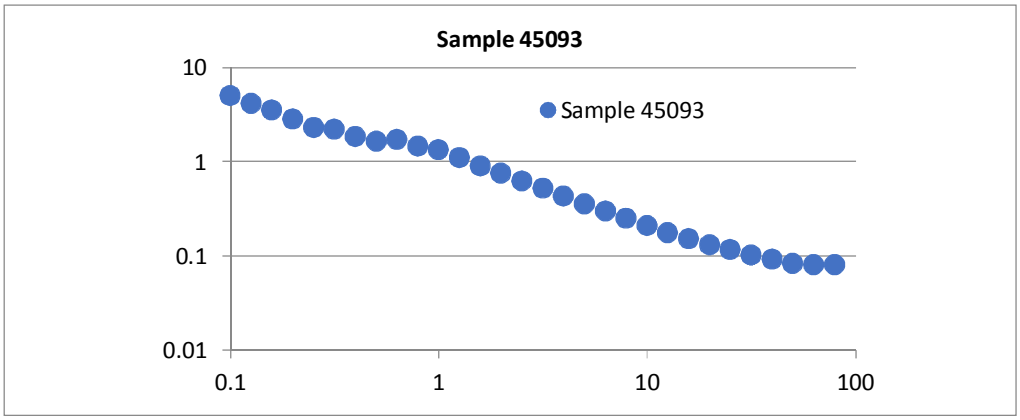

Fig. (4) Shear rate- viscosity profile for *A. tortilis* var. *spirocarpa* gum(25%w/w)

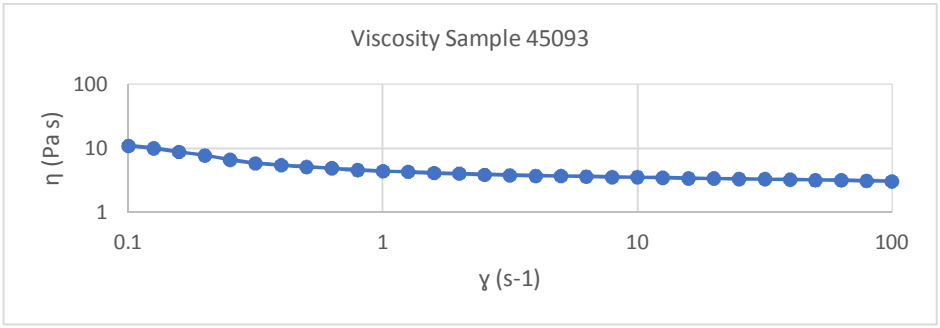

Fig. (5) Shear rate –viscosity profile for *A.tortilis* var. *spirocarpa* gum (50%w/w).

Oscillatory Rheological Properties:

Fig. 6 showed the plot of storage modulus G` and loss modulus G`` and viscosity η against frequency f(ω) at concentration (50%w/w) on applying small stress (0.1-1.003 Pa). At the lowest accessible frequencies (0.1-1Hz, 0.62- 6.28 rad/s), G` was observed to be higher than G`` due to molecular association, the response was solid-like more than viscus-like.

The viscosity curve crosslink G` curve at frequency (0.15Hz, 0.9 rad/s) and then crosslink G `curve at frequency (0.3Hz,1.9 rad/s). The dynamic viscosity (η = G``/ ω) , ω the angular frequency, then equal 2.1Pa.s and at frequency (6.28 rad/s) equal (10.4 Pa.s), the author ascribed the elastic nature in the gum to AGP micelles , on increasing the frequency it started to degrade at dynamic viscosity to give AG and GP micelles. The viscosity exhibited shear thinning with increasing frequency.

Both moduli were increased with increasing frequency. The moduli crosslink junction (Gc) and frequency (Wc) were found to be 121.9 Pa, and 2.5 Hz, 21.7rad/s respectively, which is considered as a measure of the polymer solution relaxation time. Both G` and G`` became constant and frequency independent. Then G`` was increased more than G`, independent of frequency.

The phase shift δ was (0.59), then angle was 31° lied between 0° and 45° therefore, the solution was viscoelastic solid. Complex modulus G*(Pa) started from low value 29.8 to high value 381 indicated the polymer was gradual from soft-solid to hard-solid present rigidity and integrity of the material internal structure, or cross-linking and entanglement of dissolved polymer. Complex (G*= G`/ G``). Complex G* was greater than unity, so the solution prone to hard solid. The oscillation measurement for *A. tortilis* var *raddiana* (Abdelrahman,2011) shows that G` is started higher than G`` refers also to certain rigidity in the gum structure., and exhibits solid-like behavior.

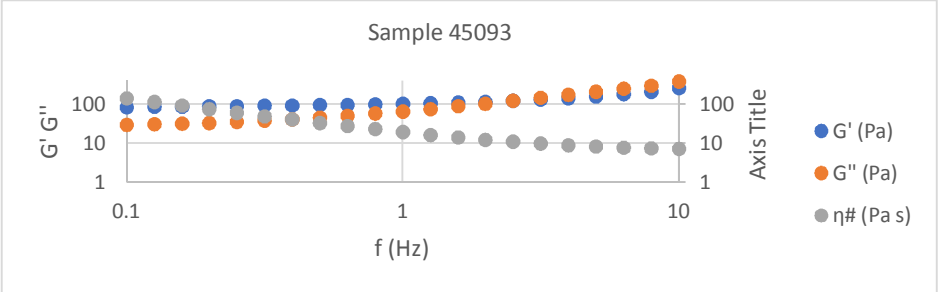

Fig. (6) Dynamic moduli for *A. tortilis* var. *spirocarpa gum*

Conclusion:

The gum from *A tortilis var spirocarpa* is grade 1 emulsifier. The behaviour of flow is Newtonian at high shear rate and at high concentration. The dynamic rheological behaviour exhibits solid- like behaviour.The other varients *A. tortilis* var *raddiana* and *A.tortilis* var *tortilis* also consider as grade 1 emulsifiers and exhibit shear thinning behavior with low concentrations and Newtonian behavior with high concentration and high shear rates. As a result, the three varients of A.tortilis.exhibit a great similarity in functional properties. .

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