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Solution and conformational properties of gum kondagogu (*Cochlospermum gossypium*) – A natural product with immense potential as a food additive

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ABSTRACT

Gum kondagogu (*Cochlospermum gossypium*), a natural biopolymer, was investigated to assess its solution and conformational properties. Solution properties of the gum were determined by light scattering and viscometry. Deacetylated gum was thermally more stable than native gum. The molecular mass of native gum ranged from $7.23 \pm 0.15 \times 10^6$ to $9.83 \pm 0.05 \times 10^5$ Da, whilst that of deacetylated gum was $3.61 \pm 0.25 \times 10^7$ to $9.77 \pm 0.08 \times 10^5$ Da. Intrinsic viscosity was observed to be $9.75 \times 10^{-4}\ M_w^{0.80}$ (dl/g) and $9.32 \times 10^{-4}\ M_w^{0.76}$ (dl/g) for native and deacetylated gum, respectively. The Mark–Houwink–Sakurada exponent (α) values of 0.80 and 0.76 for gum kondagogu (native and deacetylated) suggests that its macromolecular nature assumes a more expanded conformation and behaves as a random coil in good solvent. The structure-sensitive parameter (ρ) obtained for the gum indicates a value of 1.83, which suggests a semi-flexible conformation for gum.

1. Introduction

Exudate gums are amongst the oldest natural products exploited by mankind for their unique range of functionalities as food additives. Natural polysaccharides are biopolymers that are considered to be biocompatible and environmentally friendly. Moreover, these natural polymers are generally non-toxic, cheap and freely available compared to the synthetic polymers. Natural polysaccharides are amongst the richest renewable sources of biopolymers and have potential applications in a variety of fields that include the food, cosmetics, pharmaceutical and petroleum industries (Murphy, 1997).

Gum arabic, gum karaya and gum tragacanth are obtained as the natural exudates of different tree species and exhibit unique properties in a wide variety of food and non-food applications (Verbeken, Dierchx, & Dewettinck, 2003). Gum kondagogu (*Cochlospermum gossypium*) a tree gum from India, is also obtained as a natural exudate, and is yet to be explored for its potential applications. The Food and Agricultural Organization (FAO), Rome, had included the gum sourced from *C. gossypium* as karaya gum (*Sterculia urens*), as a recognised food additive (FAO, 1991). In the recent past, the morphological, physicochemical, structural and rheological properties of gum kondagogu have been extensively studied by us and the primary structure of this biopolymer was established to contain sugars, such as arabinose, rhamnose, glucose, galactose, mannose, glucuronic acid and galacturonic acid. This gum has been grouped under substituted rhamnogalacturonans. Based on the spectroscopic characterisation, the probable structural feature assigned to gum kondagogu was (1 → 2) β-D-Gal *p*, (1 → 6)-β-D-Gal *p*, (1 → 4) β-D-Glc *p* A, 4-0-Me-α-D-Glc *p* A, (1 → 2) α-L-Rha (Janaki & Sashidhar, 1998; Vinod, Sashidhar, Sarma, & Vijaya Saradhi, 2008; Vinod, Sashidhar, Suresh, et al., 2008). The toxicological evaluation of gum kondagogu established that this gum was non-toxic and has potential application as a food additive (Janaki & Sashidhar, 2000). Native gum kondagogu is a complex and acidic polysaccharide with high solution viscosity and gelation characteristics (Vinod, Sashidhar, Sarma, et al., 2008; Vinod, Sashidhar, Suresh, et al., 2008) such as those observed for the gum exudate obtained from *S. urens* (gum karaya) (Le Cerf, Irinei, & Muller, 1990).

Understanding the solution properties of the gum is an essential criterion for its application as an ingredient in food systems. Furthermore, the conformation of the biopolymer has a great impact on its functionality or biological activity in food processing (Li, Cui, & Wang, 2006). Earlier studies on plant exudates gums such as karaya, arabic, and tragacanth have established that, solution and conformational properties of these biopolymers are dictated by the primary structure and are crucial in understanding the structure–function relationship (Le Cerf et al., 1990; Mohammadifar, Musavi, Kiumarsi, & Williams, 2006; Verbeken et al., 2003). Additionally, the thermogravimetric analysis of polysaccharides and their derivatives have been found to be promising and sensitive techniques for characterising structural features/modifications

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and in assessing accurately the decomposition pattern and thermal stability of natural polymers (Varma, Kokane, Pathak, & Pradhan, 1997; Zohuriaan & Shokrolahi, 2004). Even though, the structural and rheological properties of gum kondagogu have been investigated in the past (Janaki & Sashidhar, 1998; Vinod, Sashidhar, Sarma, et al., 2008; Vinod, Sashidhar, Suresh, et al., 2008), the solution properties in relation to molecular conformational and macromolecular behaviour of this novel gum are yet to be established.

To study the structure–function relationships of polymers, it is essential to obtain an accurate measurement of their molecular mass. Light scattering is one of the important experimental tools for the characterisation of these properties of high molecular mass polymers, due to its high sensitivity for detecting large molecules at low concentrations (Ma, Sun, & Bloomfield, 1995). Dynamic light scattering (DLS) is an effective approach to study the aggregation behaviour of polymers in dilute solutions (Li, Wang, Cui, Burchard, & Yada, 2007).

In the present communication, we report the thermal stability of the biopolymer based on thermogravimetric analysis. The intrinsic viscosity $[\eta]$ was determined by Ubbelohde viscometer. The weight-average molar mass (M_w), second virial coefficient (A_2), and z-average radius of gyration (R_g) were established by static light scattering (SLS) analysis. Additionally, the acetyl content of the gum was determined by a ^{1}H NMR method. Furthermore, the translational diffusion coefficient (D), hydrodynamic radius (R_h) and the polymer chain conformation were investigated by dynamic light scattering (DLS) method. Light scattering and viscometric measurements formed the basis for establishing the solution properties of the biopolymer.

2. Materials and methods

2.1. Sample origin

Gum kondagogu samples were collected from Girijan Cooperative Corporation, Hyderabad, (Andhra Pradesh, India, a State Government undertaking), and gratis samples were provided by M/s D.K. Enterprises, Hyderabad, India. Gum kondagogu, Grade 1 (hand-picked, fresh, clean with no extraneous material), was used in the experimental analysis. Gum samples collected were stored in air-tight polypropylene jars in desiccated condition. Deionised (Milli-Q, reverse osmosis) water was used for all experimental studies. All other chemicals used were of analytical reagent grade.

2.2. Moisture and acetyl content

Moisture content of native gum kodagogu was determined by the standard method of AOAC (AOAC, 1984). The acetyl content in the native gum kondagogu was determined by an earlier reported method based on ^{1}H NMR (Bedouet, Courtois, & Courtois, 2003). Briefly, ^{1}H NMR spectra (Varian, UNITY-400, Steinhausen, Switzerland) of native gum kondagogu (10 mg/ml) dissolved in D_2O were obtained and chemical shifts were reported in ppm relative to an internal standard TSP (3-(trimethylsilyl) propionic-2,2,3,3,-d_4 acid, sodium salt, 98% D) and recorded at 80 °C. To this solution, 0.001 mol of NaOH were added and 256 scans were recorded after dissolution.

2.3. Preparation of native and deacetylated polysaccharide

Gum kondagogu was powdered in a high-speed mechanical blender, (Philips, Mumbai, India) and later sieved using a bin (mesh size – 250 μm), so as to obtain a fine and uniform sample. Gum kondagogu powder (1 g) was accurately weighed and dispensed into a clean glass beaker containing one litre of deionised water. The entire gum solution was kept on a magnetic stirrer at room temperature and gently stirred overnight. Later, the gum solution was allowed to stand at room temperature (30 °C) for 12 h, so as to separate any undissolved matter, The gum solution was filtered through a #G-2 sintered glass funnel followed by a #G-4 sintered funnel (Le Cerf et al., 1990). The clear solution so obtained was freeze-dried (Dry Winner, Heto-Holten, A/S, DK-3450, Allerd, Denmark) and stored until further use. This processed sample was designated as ‘native’ polysaccharide.

Deacetylated gum kondagogu was prepared by an earlier reported method (Lee, Ashby, & Day, 1996). Briefly, gum kondagogu powder (1 g) was accurately weighed and dispensed into a clean glass beaker containing one litre of deionised water. Three volumes of this solution were mixed with one volume of 1 M NaOH solution. After incubation for 20 min at room temperature with gentle agitation on a magnetic stirrer, one volume of 1 M HCl was added to neutralise the solution (to a final pH of 7.0). The deacetylated gum kondagogu was dialysed extensively against Milli-Q water to remove residual salts. The gum solution was filtered through a #G-2 sintered glass funnel followed by a #G-4 sintered funnel (Le Cerf et al., 1990). The clear solution so obtained was freeze-dried and stored until further use. Deacetylation was monitored by infrared spectroscopy (Vinod, Sashidhar, Sarma, et al., 2008).

2.4. Sample preparation for light scattering and viscometric measurements

Stock solutions of 10 mg/ml of native and deacetylated gum kondagogu samples were prepared in 1.0 M NaCl aqueous solutions. All of the solutions were filtered through a 0.45 μm nylon syringe filter. The required concentrations of solutions of native and deacetylated gum kondagogu were prepared from the stock solutions for light scattering and viscometric measurements. Solvent (1.0 M NaCl solution) was filtered through Durapore (Millipore) 0.22 μm membrane, whilst the gum solutions were filtered through Durapore 0.45 μm membrane. All solution preparation stages were carried out in a laminar airflow cabinet to minimise particulate contamination.

Native and deacetylated gum kondagogu ranging from 0.2 to 1.0 mg/ml (w/v) in 1.0 M NaCl was used for SLS study. Concentrations ranging from 0.1 to 1.0 mg/ml of fractionated gum kondagogu (native and deacetylated) sample were prepared in 1.0 M NaCl aqueous solutions and used for viscometric analysis.

2.5. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed using both native and deacetylated gum samples (freeze-dried). Gum sample (5 mg) taken in a platinum pan was subsequently subjected to thermogravimetric analysis under a dynamic nitrogen atmosphere between 25 and 700 °C (Mettler Toledo Star System, Columbus, OH). The heating rate was uniform at 10 °C/min.

2.6. Ultrasonication

Gum kondagogu was fractionated into different molecular mass fractions by ultrasonication. Ultrasonication procedure was selected as the best means for producing several fractionated samples of different molecular weights without any structural or chemical modification, as reported earlier (Suslick & Price, 1999). Sample solutions were exposed to 400 W of 20 kHz frequency sound with an ultrasonicator (Vibra Cell, VCX 400, Danbury, CT) for various time points (0, 1, 3 and 6 min), so as to yield a desired degree of chain scission. The temperature was maintained at ~10 °C by cooling.

2.7. Intrinsic viscosity [η]

The intrinsic viscosity measurements were performed in an Ubbelohde viscometer (Schott Gerate AVS 350, Mainz, Germany) immersed in a constant temperature water bath to maintain the temperature at 25 ± 0.01 °C. Stock solutions (0.1% w/v) of native and deacetylated gum were prepared in 1.0 M NaCl. Capillary No. 52501/ 0B, at a flow time of 161.25 seconds (average of five measurements) for 1.0 M NaCl, was used for the experimental studies. The kinetic energy correction was made according to Hagenbach correction table (supplied along with the instrument), corresponding to the capillary used for measurement. The Zeta (ζ) potential of the gum solutions was measured before and after the addition of NaCl (Malvern Zetasizer Nanosystem, Malvern, UK). Ultra-pure sodium chloride was used for the preparation of gum solution, in order to overcome the polyelectrolytic effect of gum in pure water (Vinod, Sashidhar, Suresh, et al., 2008). Concentrations ranging from 0.1 to 1.0 mg/ml of gum were prepared in 1.0 M NaCl aqueous solutions. The flow time, t, was measured at different concentrations and relative viscosity was determined from $\eta_{rel} = t/t_o$, where, t_o is the flow time for pure solvent. The intrinsic viscosity [η] was calculated using the following relationship:

$$[\eta] = \lim_{c\to 0}(\eta_{sp}/c) = \lim_{c\to 0}(\ln \eta_r/c), \tag{1}$$

where c is the concentration of polymer, η_r is the relative viscosity; η_{sp} is the specific viscosity, defined as $\eta_r - 1$. Huggins–Kramer plots of η_{sp}/c and $\ln(\eta_r)/c$ versus c were then used to estimate the intrinsic viscosity [η] by extrapolation to zero concentration.

2.8. Light scattering studies

SLS and DLS experiments were performed on PhotoCor FC-complex (*Photocor Instruments*, *College Park*, MD, USA) set-up equipped with the real time multiple τ correlators, a photomultiplier and an automatic goniometer. A Uniphase 1135P He–Ne laser (λ = 633 nm, 20 mW power) was used as a light source. Total intensity light scattering measurements were conducted in the angular range of 30–140° for static and 90° for dynamic light scattering. Ultra-pure toluene was used as a reference with a Rayleigh ratio of 1.398×10^{-5}/cm at 298.15 K in the static light scattering mode. The refractive index increment dn/dc, was determined as 0.140 ml/g for gum kondagogu in 1.0 M NaCl solutions (Vinod, Sashidhar, Suresh, et al., 2008). The particle size distributions were calculated by the constrained regularisation (CONTIN) programme, based on the inverse Laplace transformation method, using Photocor-FC dynamic light scattering software (supplied by the instrument manufacturer). All the light scattering measurements were performed at 25 °C. Ultra-pure sodium chloride (1.0 M) solution was used as the solvent.

2.9. Determination molecular weight by static light scattering (SLS)

Static light scattering measures the average total scattering intensity over a selected time period. It provides a convenient method for deriving several molecular parameters simultaneously, including weight-average molecular mass (M_w), second virial coefficient (A_2) and radius of gyration (R_g) based on the following equation:

$$Kc/R\theta = 1/M_w + 1/3(R_g^2/M_w)q^2 + 2A_2c, \tag{2}$$

where, K is an optical contrast factor, c is the polymer concentration, $R\theta$ is the Rayleigh ratio (normalised scattering intensity). For dilute solutions, D_{app} measured at a finite scattering angle is related to the sample concentration (C) and scattering angle θ by equation,

$$D_{\text{app}} = D(1 + k_dC)1 + (fR_g^2q^2), \tag{2.1}$$

where, q (magnitude of scattering vector) is defined by the following equation, k_d is the diffusion second virial coefficient, f is the dimensionless number and D is the translational diffusion coefficient. The scattering vector (q) is defined by the following equation:

$$q = (4\pi/\lambda)\sin(\theta/2), \tag{2.2}$$

where $\lambda = \lambda_0/n_0$, the wavelength of the light in a medium of refractive index n_0, λ_0, the wavelength in a vacuum. The optical contrast factor K is given by the following equation:

$$K = 4\pi^2(dn/dc)^2n_0^2/(N_0\lambda_0^4). \tag{2.3}$$

Where dn/dc is the refractive index increment of the solution and N_0 is the Avogadro constant. Berry plot is a graphical technique to extrapolate simultaneously $\sqrt{[K_c/R(\theta)]}$ to zero angle and infinite dilution. A Berry plot involves constructing a plot of $\sqrt{[K_c/R(\theta)]}$ against $\sin^2(\theta/2) + kc$ and fitting polynomial in $\sin^2(\theta/2)$ to the data (Berry, 1966). From the plot of $\sqrt{[K_c/R(\theta)]}$ versus $\sin^2(\theta/2) + kc$ and by extrapolating to zero angle concentration, R_g, A_2 can be obtained from the slopes and M_w is given by the inverse of the intercept.

2.10. Dynamic and static light scattering (DLS)

Dynamic light scattering measures the fluctuation of the intensity over time through the determination of a time-dependent auto-correlation function. An appropriate mathematical approach has to be used to extract meaningful physical parameters. The homodyne intensity correlation function $G^{(2)}(\tau)$ was measured within the range of delay times from 2×10^{-8} to 5×10^3 s. The normalised homodyne intensity correlation function $g^{(2)}(\tau)$ is related to the normalised electric field time correlation function $g^{(1)}(\tau)$ by the Siegert relation (Stepanek, 1993):

$$g^{(2)}(\tau) = 1 + \beta\left|g^{(1)}(\tau)\right|^2, \tag{3}$$

where, $g^{(2)}(\tau) = G^{(2)}(\tau)/G^{(2)}(\propto)$, $G^{(2)}(\propto)$ is an experimentally-determined baseline, β is a coherence factor. In dilute solutions of monodisperse particles, field correlation function is connected with the translation diffusion as follows:

$$g^{(1)}(\tau) = \exp(-t/\tau) = \exp(-\Gamma t) = \exp(-Dq^2t), \tag{3.1}$$

where $D = \lim_{q\to 0}(\Gamma/q^2)$.

Here, τ is the decay time, $\Gamma = 1/\tau$ is the decay rate, D is the diffusion coefficient, $q = (4\pi/\lambda)\sin(\theta/2)$ is the wave vector, (θ) is the scattering angle. If there is a large number of independent decay processes in the system, $g^{(1)}(\tau)$ is a weighed sum of individual contributions. In continuous form, the equation can be re-written as:

$$g^{(1)}(\tau) = \int_o^\infty G(\Gamma)\exp(-\Gamma\tau)d\Gamma, \tag{3.2}$$

where $G(\Gamma)$ is a distribution of scattered light over decay times. Constrained regularisation programme by Provencher (1982) (CONTIN) based on the inverse Laplace transform was used for determination of this distribution function. In the case of a polydisperse solute, the distribution of the diffusion coefficient, and the hydrodynamic diameter distribution, was obtained from $g^{(1)}(\tau)$ by inverse Laplace transformation using CONTIN method. For every relaxation maximum found in these spectra of decay times, translational diffusion coefficient D and hydrodynamic radius R_h were calculated using the following relationship:

$$D = 1/q^2\tau, D = kT/6\pi\eta R_h, \tag{3.3}$$

wherein η is the solvent viscosity. The true value of D and R_h were obtained by extrapolation to $q = 0$ and $c = 0$. The hydrodynamic radius (R_h) was calculated by applying the Stokes–Einstein relation for sphere particles, given by the following equation:

$$R_h = kT/6\pi\eta D, \tag{3.4}$$

where T is the absolute temperature, k is the Boltzmann constant and η is the solvent viscosity.

2.11. Determination of Mark–Houwink–Sakurada constants

Native and deacetylated gum samples with a range of molecular weight distributions were prepared by ultrasonication. M_w (weight-average molecular mass) and intrinsic viscosity $[\eta]$ values obtained for the native and deacetylated gum kondagogu solutions were used to plot log $[\eta]$ against log M_w, to yield a straight line. From the plot K and α values were determined. The weight-average molecular mass of the deacetylated gum was determined by the Mark–Houwink–Sakurada equation, given by,

$$[\eta] = KM_w^{\alpha}, \tag{4}$$

where M_w is the weight-average molecular weight and the parameters K and α reflect a combination of contributions, but K is related essentially to the intrinsic or local chain flexibility, including the orientation of the bonds to and from the constituent monosaccharides, whereas the Mark–Houwink–Sakurada exponent (α) can reflect the chain geometry, branched, sphere, rod or coil, and are related to local stiffness of the polymer and depend on the nature of the polymer, solvent and temperature.

3. Results and discussion

3.1. Moisture and acetyl content

The moisture content of native gum kondagogu was observed to be 15.5 ± 1.12% (w/w). Acetyl content of the gum was found to be 12% (w/w), as determined by ^{1}H NMR analysis. The acetyl content of native gum kondagogu was found to be higher than the reported values for gum karaya (8%) and *S. striata* (10.7%) (Brito, Silva, De Paula, & Feitosa, 2004). The presence of acetyl groups has relevance in the stability of the junction zones and water solubility of the native gum, as it has been established that deacetylated gums (karaya gum and *S. striata* gum) show an increased water solubility and assumes a more expanded and less branched confirmation (Le Cerf et al., 1990; Silva, Brito, de Paula, Feitosa, & Paula, 2003).

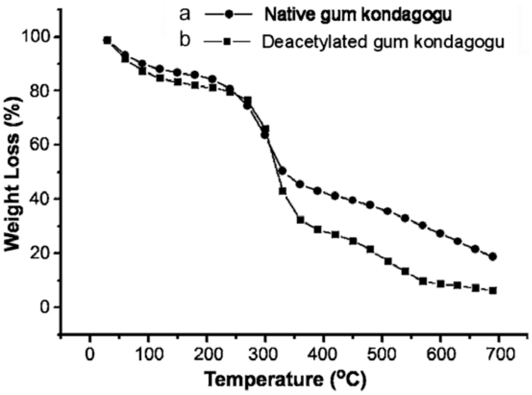

Fig. 1. TGA thermograms of: (a) native and (b) deacetylated gum kondagogu. Analysis carried out under dynamic N_2 atmosphere in the temperature range of 25–700 °C, with heating rate of 10 °C/min.

3.2. TGA analysis

Thermogravimetric analysis is a simple and accurate method for studying the decomposition pattern and the thermal stability of polymers. Thermogravimetric analysis of gum kondagogu was carried out, in the range 25–700 °C, to determine thermal stability and to assess the volatile components of the gum by monitoring the weight change that occurs, during the thermal processing of the gum. The TGA thermograms of native and deacetylated gum kondagogu obtained at a scan rate of 10 °C/min in nitrogen atmosphere are shown in Fig. 1a and b. In the case of native gum kondagogu, three distinct zones are observed where the weight loss was observed. The early minor weight loss (16.76%) in the sample can be attributed to the desorption of water molecules (as water hydrogen-bound to the saccharide structure). The second zone, i.e., the degradation zone at 210–390 °C, where a major weight loss (53%) was observed, which can be attributed to the decomposition of the polymer. In the last zone at 420–690 °C, the weight loss was observed to be 20.99%. In the case of deacetylated gum kondagogu, the weight losses were observed to be 12.03% (at 30–150 °C), 41.75% (at 210–390 °C) and 22.84% (at 420–690 °C), respectively. Interestingly, the decomposition pattern of deacetylated gum kondagogu was distinctly different from that of native gum kondagogu, suggesting that the deacetylated form of gum was more thermally stable than its native form. Earlier, similar behaviour was also observed in guar gum and its cross-linked derivatives (Varma et al., 1997) Guar gum (native) was less stable than its cross-linked products and the degradation observed was 80% at 320 °C. The thermal stability of gum arabic was observed to be

Table 1
Weight-average molecular weight (M_w), second virial coefficient (A_2) and radius of gyration (R_g) of native and deacetylated gum kondagogu as determined by static light scattering and Berry plot.

Sample[a]	Sonication time (min)	M_w (g/mole)[b]	A_2 (cm^3 mol/g^2)	R_g (nm)
Gum kondagogu				
Native form	0	$7.23 \pm 0.15 \times 10^6$	2.75×10^{-5}	229.86 ± 1.5
	1	$2.69 \pm 0.10 \times 10^6$	2.10×10^{-4}	120.81 ± 1.2
	3	$1.12 \pm 0.09 \times 10^6$	1.18×10^{-4}	104.68 ± 1.2
	6	$9.83 \pm 0.05 \times 10^5$	3.24×10^{-4}	66.74 ± 0.85
Deacetylated form	0	$3.61 \pm 0.25 \times 10^7$	3.29×10^{-5}	289.5 ± 2.2
	1	$2.47 \pm 0.12 \times 10^6$	1.02×10^{-4}	160.06 ± 1.5
	3	$1.62 \pm 0.11 \times 10^6$	1.39×10^{-4}	103.54 ± 1.4
	6	$9.77 \pm 0.08 \times 10^5$	3.08×10^{-4}	81.74 ± 0.84

[a] Corrected for moisture content.
[b] Values based on four independent experiments.

Table 2
Experimental data of dynamic light scattering and viscometry of native and deacetylated gum kondagogu fractions.

Sample[a]	Sonication time (min)	$[\eta]$ (dl/g)[b]	D (cm^2/s) $\times 10^{-8}$	R_h (nm)
Gum kondagogu				
Native form	0	32.68 ± 0.22	1.83	123.58 ± 0.85
	1	13.11 ± 0.02	3.47	65.30 ± 0.56
	3	7.91 ± 0.05	3.90	57.51 ± 0.62
	6	6.01 ± 0.02	6.29	36.07 ± 0.18
Deacetylated form	0	59.34 ± 1.38	1.45	155.64 ± 1.50
	1	10.04 ± 0.08	2.62	56.27 ± 0.95
	3	5.56 ± 0.03	4.03	46.51 ± 0.82
	6	3.50 ± 0.06	5.08	44.66 ± 0.58

η – Intrinsic viscosity; D – translational diffusion coefficient; R_h – hydrodynamic radius.
[a] Corrected for moisture content.
[b] Values based on four independent experiments.

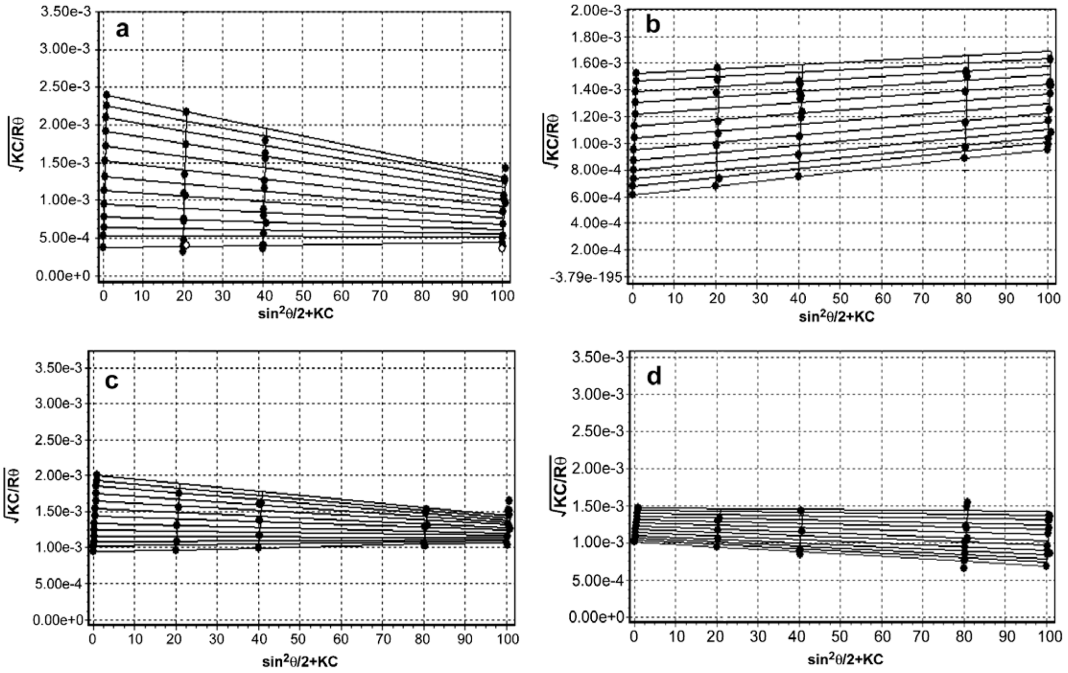

Fig. 2. Berry plot (based on static light scattering data) of native (a–d) gum kondagogu of different sonicated fractions (0, 1, 3 and 6 min) in 1.0 M NaCl aqueous solutions at 298 K. Measurement angles range from 30° to 140°; Concentrations range 0.2–1.0 mg/ml.

lower than gum tragacanth (Zohuriaan & Shokrolahi, 2004). Gum kondagogu and its deacetylated derivative have higher thermal stability. This may be a unique property of this gum in relation to its molecular composition (a highly acidic biopolymer compared to other polysaccharides (Vinod, Sashidhar, Sarma, et al., 2008), resulting in its higher thermal stability.

3.3. Intrinsic viscosity [η]

Intrinsic viscosity [η] values of ultrasonicated samples (0, 1, 3 and 6 min) of native and deacetylated gum kondagogu in 1.0 M aqueous NaCl were found to be 32.68 ± 0.22, 13.11 ± 0.02, 7.91 ± 0.05, 6.0 ± 0.02 and 59.34 ± 1.38, 10.04 ± 0.08, 5.56 ± 0.03

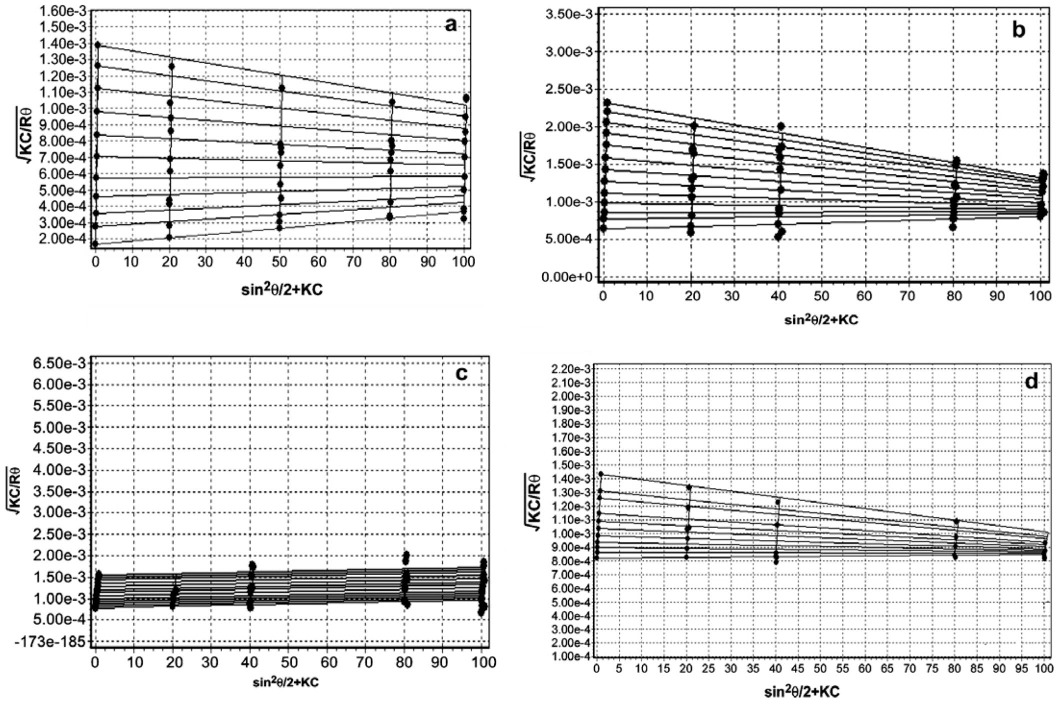

Fig. 3. Berry plot (based on static light scattering data) of deacetylated gum kondagogu (a–d) of different sonicated fractions (0, 1, 3 and 6 min) in 1.0 M NaCl aqueous solutions at 298 K. Measurement angles range from 30° to 140°; Concentrations range 0.2–1.0 mg /ml.

and 3.5 ± 0.06 dl/g, respectively (Table 2). The intrinsic viscosity values were used to determine α and K values by the Mark–Houwink–Sakurada equation (Fig. 4). The intrinsic viscosity for this gum was observed to be greater than that of gum karaya and other tree gums, indicating the molecular mass of the gum kondagogu to be higher than the other tree biopolymers (Verbeken et al., 2003).

3.4. Static light scattering studies

Berry plots of ultrasonicated (different time points) native and deacetylated gum kondagogu at different concentrations were measured by static light scattering, are shown in panels Figs. 2a–d and 3a–d. The weight-average molecular mass (M_w), radius of gyration (R_g) and second virial coefficient (A_2) extracted from Berry plots are listed in Table 1. The positive values of the second virial coefficients of the fraction indicated that NaCl solution (1 M) to be a good solvent for the native and deacetylated gum kondagogu. The polyelectrolytic effect of the gum in NaCl (1.0 M) was completely eliminated and linear plots were obtained with positive slopes at respective concentrations for both native and deacetylated gum samples, as depicted in Berry plots Figs. 2 and 3a–d. The Berry curve fitting method relies on constructing a plot of $[K_c/\mathrm{R}(\theta)]^{1/2}$ against $\sin^2(\theta/2)$ and fitting polynomial in $\sin^2(\theta/2)$ to the data. Berry plot was preferred over the Zimm plot, as molecules with large R_g values of >50 nm, were observed for gum kondagogu (Berry, 1966). Berry plots (Figs. 2 and 3) indicate that the angular dependence of the scattered light did not change systematically with decreasing concentration of the polymer, suggesting no detectable configurational change of the molecules upon dilution, signifying that there was no expansion or contraction of the polymer. This behaviour may be explained on the basis of negating the polyelectrolytic character of the gum in the presence of 1.0 M sodium chloride (Vinod, Sashidhar, Suresh, et al., 2008). The values of R_g were observed to increase with increase in molecular mass and were higher than that of R_h in both, native and deacetylated gum kondagogu (Tables 1 and 2). The relationship between R_g and molecular mass (M_w) or R_h can be used to interpret the shape of gum kondagogu chains in 1.0 M NaCl solutions.

Previous studies on gum karaya have indicated that the molecular mass of native and deacetylated gum to be 2–5 × 10^6 and 12–16 × 10^6 Da, respectively (Le Cerf et al., 1990). The experimental data of the present investigation shows that native and deacetylated gum kondagogu have molecular masses of 7.23 ± 0.15 × 10^6 and 3.61 ± 0.25 × 10^7 Da, respectively (non-sonicated sample). Deacetylation leads to increased solubilisation and gelation of material, resulting in higher molecular mass with a greater intrinsic viscosity as compared to its native form. A similar observation was made earlier with respect to gum karaya, wherein the deacetylated form had higher molecular mass (Le Cerf et al., 1990). Possibly the removal of acetyl groups results in decreased polymer chain interactions. In comparison to the other tree gums, the molecular mass of gum kondagogu was found to be higher than the molecular mass of gum karaya (native and deacetylated forms) (Le Cerf et al., 1990), gum tragacanth (Mohammadifar et al., 2006), gum Arabic (Randall, Phillips, & Williams, 1989; Vabdevelde & Frenyo, 1985) and guar gum (Wang, Fllis, & Ross-Murphy, 2003) as reported in the literature. This property of gum kondagogu makes it a promising viscosity enhancer, as the higher molecular mass of the gum directly relates to its intrinsic viscosity.

3.5. Dynamic Light scattering studies

The conformational changes of gum kondagogu were also studied by dynamic light scattering. Applying the Stokes-Einstein equation, the hydrodynamic radii, R_h, were calculated. The hydrodynamic radius of each sonicated fraction of native and deacetylated gum kondagogu at a specific angle of 90° was calculated by the CONTIN method and is shown in Table 2. The polydispersity indices determined by dynamic light scattering of fractions obtained from different sonication times (0–6 min) of native gum were 1.85, 1.23, 0.911 and 0.77. For deacetylated gum they were 1.65, 1.23, 1.01 and 0.805. The theoretical value of the polydispersity index is reported as 2.0 for random degradation of infinite chains (Tanford, 1961). Very high values of polydispersity index, as determined by SLS and DLS, are not appropriate for the prediction of molecular conformation (Li et al., 2006). Earlier, many interpretation methods for auto-correlation functions of DLS measurements were developed, based on different algorithms (Wang, Huang, Nakamura, Burchard, & Hallett, 2005). Amongst these, cumulants and CONTIN methods are commonly employed for DLS data analysis. In the present communication CONTIN method was preferred because this method gives accurate values of the average and variance (Li et al., 2006).

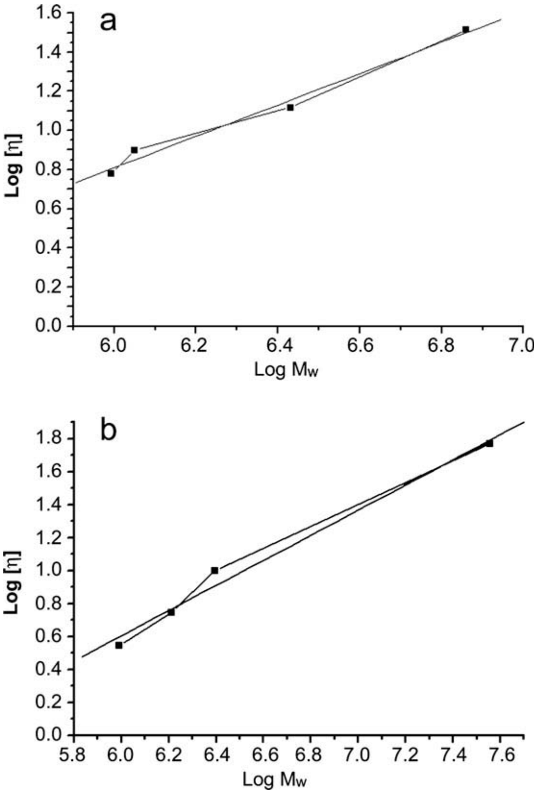

Fig. 4. Double logarithmic representations of the molecular weight versus the intrinsic viscosity of fractionated (a) native and (b) deacetylated gum kondagogu in 1.0 M aqueous NaCl solutions. The slope of the line gives the value of α and the antilog of y-intercept indicates the value of K according to Mark–Houwink–Sakurada equation. The straight line indicates a linear least-square fit.

3.6. Molecular shape

As static and hydrodynamic dimensions vary characteristically with the structure of the macromolecules, a combination of the two may provide qualitative information on the architecture of the macromolecules. The structure-sensitive parameter ρ is defined as the ratio of radius of gyration R_g, obtained from static light scattering, to the hydrodynamic radius R_h from dynamic light scattering studies. R_g/R_h ratio is known to depend on polymer architecture, chain conformation and polydispersity of the polymer in solution. The relationships between R_g, R_h and M_w of polymers and the $\rho = R_g/R_h$ value, provide information on molecular shape and conformation of the polymers in solution. The structure-sensitive parameter (ρ) obtained for gum kondagogu was observed to be 1.83, which suggests a semi-flexible conformation for gum kondagogu in 1.0 M NaCl solution. A similar semi-flexible chain character was previously reported for gum tragacanth (Mohammadifar et al., 2006). Contemporary literature survey indicates that for monodispersed random coils, the value of $\rho = 1.5$ in a θ solvent, $\rho = 1.78$ in a good solvent and $\rho > 2$ for rigid rod configurations, have been assigned (Li et al., 2006; Tanford, 1961).

3.7. Mark–Houwink–Sakurada equation

Based, on the respective M_w and intrinsic viscosity $[\eta]$ values of native and deacetylated, sonicated gum kondagogu samples, a plot of log $[\eta]$ against log M_w yielded a straight line (Fig. 4a and b). From the plot, $K = 9.75 \times 10^{-4}$, $K = 9.32 \times 10^{-4}$ and $\alpha = 0.80$, 0.76, for native and deacetylated gum, respectively, were derived and substituted in the Mark–Houwink equation. From the equation the intrinsic viscosity $[\eta]$ was calculated to be $9.759 \times 10^{-4}\ M_w^{0.80}$ (dl/g) for native gum and $9.328 \times 10^{-4}\ M_w^{0.76}$ (dl/g) for deacetylated gum. The Mark–Houwink–Sakurada exponent (α) is related to the shape of the macromolecule and has an intimate relationship with the degree of rigidity and solvation ability of a polymer. The α values of 0.80 and 0.76 for gum kondagogu (native and deacetylated, respectively) suggests that its macromolecular nature assumes a more expanded conformation and behaves as random coil in a good solvent (NaCl, 1 M solution). Earlier, reports suggest that an α value of 0.5–0.8 is indicative of linear random coil structure, whilst a value of ~1.8 shows a rigid rod structure for the polymers (Robinson, Ross-Murphy, & Morris, 1982). However, Teraoka (2001) suggested a value of α around 0.7–0.8 represents a flexible chain structure in a good solvent and a value of >1.0 for rigid chains. For gum tragacanthin, an α value of 0.87 was reported in 0.1 M NaCl and the polymer was shown to exist as a semi-flexible coil polymer (Mohammadifar et al., 2006). A compact globular structure for the polymer was reported for native gum karaya, where the α value was determined to be 0.4 (Le Cerf et al., 1990).

4. Conclusion

The decomposition pattern of deacetylated gum kondagogu was distinctly different from that of native gum, signifying that deacetylated gum was more thermally stable than the native form. The acetyl content of the native gum was found to be higher than karaya gum. The molecular parameters, such as M_w, R_g, R_h, and η of native and deacetylated and sonicated fractions, were obtained based on static, dynamic light scattering and viscometric methods. From these investigations it was concluded that the macromolecular nature of gum kondagogu assumes a more expanded conformation and behaves as random coil in good solvent (NaCl, 1 M solution). Further, the weight-average molecular mass of native and deacetylated gum, based on Berry plot, was found to be $7.23 \pm 0.15 \times 10^6$ and $3.61 \pm 0.25 \times 10^7$ Da, respectively (for 0 time sonicated sample). The intrinsic viscosity of the native and deacetylated gum kondagogu was established as $[\eta] = 9.75 \times 10^{-4}\ M_w^{0.80}$ and $9.32 \times 10^{-4}\ M_w^{0.76}$ (dl/g). It is worthwhile to note that the molecular mass and intrinsic viscosity of gum kondagogu (native as well as deacetylated form) are much higher than the values reported for the other tree gums such as karaya, tragacanth, arabic and guar gum, that are currently available in the world market. The unique physicochemical properties (Janaki & Sashidhar, 1998; Vinod, Sashidhar, Suresh, et al., 2008) of gum kondagogu that include: (i) high uronic acid content (Vinod, Sashidhar, Sarma, et al., 2008), (ii) hydrogel property, (iii) viscoelastic behaviour (Vinod, Sashidhar, Sarma, et al., 2008, (iv) high intrinsic viscosity and molecular mass and (v) high porosity, are yet to be explored by the food, textile and pharmaceutical industry.

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