Molecular Parameters and Intrinsic Viscosity of Nettle Seed (Urtica Pilulifera) Gum as a Function of Temperature

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Research Article

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Molecular parameters and intrinsic viscosity of Nettle seed (*Urtica pilulifera*) gum as a function of temperature

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This research focused to determine some molecular properties of Nettle seed gum (NSG), as one of novel and natural source of hydrocolloids, at various temperatures (10, 25, 40, 55, and 70°C) in the dilute region. The results displayed that among the models studied, the Higiro 2 model with highest R^2 and lowest RMSE values was the most proper model for determining the intrinsic viscosity of the NSG. According to this model, the intrinsic viscosity value of NSG was obtained in the range of 0.15–0.21 dl/g. It was also revealed that, as the temperature raised, the intrinsic viscosity of NSG declined. The shape factor of NSG at 40°C was spherical, however, with increasing the temperature from 40°C to 70°C, it was changed to an ellipsoidal shape. Berry number and master curve slope revealed that NSG solution at all temperatures was within the dilute regime and that no molecular entanglements were present. The parameter *b* values acquired for NSG at the intended temperatures showed that the molecular conformation of NSG was random coil. The activation energy and chain flexibility parameter calculated for NSG at the studied temperatures were 0.488×10^7 J/kgmol and 587.2, respectively.

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Urtica pilulifera L. is a member of the *Urticaceae* family[1]. *Urtica pilulifera* L. plant grows widely in areas with the Mediterranean climate[2]. This plant has been applied for the treatment of gastritis, urinary infections, and enteritis, rheumatism, liver inflammation, also skin diseases[3].

When *Urtica pilulifera* seeds are placed in water, the mucilaginous layer around the seeds absorb water and swell. Recently, the optimal conditions for extraction of Nettle seed (*Urtica pilulifera*) mucilage (NSG) were identified by the response surface methodology (RSM)[4]. Also, some physicochemical, rheological, and functional properties of NSG as a novel and natural source of hydrocolloids has been recently perused[5]. The chemical compositions of NSG were reported as follows: moisture, 9.50%; ash, 15.30%; protein, 10.42%; lipids, 1.02%; carbohydrate, 63.09% and uronic acids, 10.75%, indicating the polyelectrolyte nature of this hydrocolloid. The constituent sugars of NSG were composed of rhamnose (13.98%), arabinose (0.02%), galactose (2.99%), glucose (2.61%), xylose (0.07%), and galacturonic acid (24.71%). Besides, the effects of shear rate (14–400 s^{-1}), concentration (3-5%), and temperature (10-70°C) on the rheological properties of NSG were studied and modelled. It was also revealed that the NSG can be used as an emulsion/foam stabilizer in the food and pharmaceutical systems[5].

Viscosity estimation in the dilute solution regime, in which individual polysaccharide coils are well separated from each other, and are free to move independently, is crucial in providing the information on how molecules behave in solution and the way the structure and fundamental properties of biopolymers are related[6]. The intrinsic viscosity, [η], is a characteristic of macromolecules that directly depends on their ability to disturb the flow and indirectly on the size and shape of the molecules[7]. Attractive and repulsion interactions between macromolecules chains affect the hydration and as a result, the hydrodynamic volume of the molecules, the hydrodynamic volume is mainly related to the conformation of macromolecules in solution. The changes in the molecular parameters such as hydrodynamic volume, conformation, shape, and macromolecular entanglements can be witnessed by variations in the intrinsic viscosity[8]. Many researches have been done to determine the intrinsic viscosity of different hydrocolloids[9-16]. The molecular parameters including solution viscosities, molecular shape & conformation, voluminosity, and etc. seem to be beneficial to understand and predict the functional properties of hydrocolloids in the semi-dilute solution regime at various conditions like temperature, pH, and etc. Due to the diversity in biopolymers' structure and also external conditions in the fluid flow systems, the dilute solution properties are greatly dependent to the selected biopolymer[15]. The literature review displays the absence of perfect and enough data concerning this emerging hydrocolloid in the dilute solution domain. Therefore, this research aimed at the effect of temperature (at the levels of 10, 25, 40, 55, and 70◦C) on some dilute solution properties (intrinsic viscosity; voluminosity, and shape factor; molecular conformation; activation energy and chain flexibility parameter) of the NSG to evaluate its potentials in the food and pharmaceutical systems.

Materials and methods

Sample preparation. Nettle seed (*Urtica pilulifera*) gum was produced under the optimized conditions, as expressed by Zamani et al.[4]. Nettle seeds were soaked in the distilled water at a water/seed ratio of 40:1 and a set temperature of 59±1.0 ◦C for a period of 3.4 h (204 min). Then, the mucilage was extracted from the swelled seeds by a lab-scale extractor (model 402, Pars-Khazar Com., Iran) and dried in a forced-convention laboratory oven (model 4567, Kimya Pars Com., Iran) at 36 ◦C, milled and sieved by applying a mesh 18 sifter. NSG powders were packaged in seal bags and maintained in a cool and dry place for further measurements.

Estimation of intrinsic viscosity. Gum solutions (2.5 g/dl) were prepared by dispersing the NSG powder in deionized water. The samples were then stirred (500 rpm) with a magnetic stirrer for 60 min at 25 ∘C until complete dissolution, and the solutions were placed in a refrigerator at 4 ∘C for 24 h to complete the hydration. The dynamic viscosity of the NSG solutions (η) and the solvent (η_s¸ deionized water) were measured at three replications in a thermostatic water bath, using an Ubbelohde capillary viscometer (Cannon Instrument, USA; capillary tube No. 100, K=0.019908 mm^2/s^2). The measurements were performed at five temperature levels of 10, 25, 40, 55, and 70∘C. To obtain the dynamic viscosity of the samples, the time of passage between two lines marker of the viscometer was recorded. Data were used to calculate the relative viscosity (η_{rel}) and specific viscosity (η_{sp}) using the following relationships:

$$\eta_{rel} = \eta/\eta_s \tag{1}$$

$$\eta_{sp} = \eta - \frac{\eta_s}{\eta_s} = \eta_{rel} - 1 \tag{2}$$

The intrinsic viscosity [η] is often estimated using the Huggins equation (3) and Kraemer equation (4) models. Based on these models, the reduced viscosity (η_{sp}/C) and inherent viscosity ($ln\eta_{rel}/C$) data versus the gum concentration were plotted and then the curves were extrapolated to zero concentration. The intercepts were considered as the intrinsic viscosity[17].

Huggins' equation[18]:

$$\frac{\eta_{sp}}{C} = [\eta] + K_H[\eta]^2\, C \tag{3}$$

Kraemer's equation[19]:

$$\frac{ln\eta_{rel}}{C} = [\eta] + K_K[\eta]^2C \tag{4}$$

where, k_H, k_k, and C are the Huggins constant, the Kraemer constant; and the gum concentration, respectively. McMillan et al.[20] stated that methods of intrinsic viscosity determination based on slopes of plots (e.g. Tanglertpaibul-Rao's model equation (5) and Higiro's models equations (6), and (7) have a higher correlation coefficient and a lower standard error than the methods based on the extrapolation (e.g. Huggins and Kraemer models). Nickerson et al.[21] also stated that since polymer concentrations are provided by sequential dilution, the error in the expression (η_{sp}/C) increases, and the data fitting to the Huggins model is difficult. Therefore, in the present study, three following slope-based models were used to estimate the intrinsic viscosity of the samples.

Tanglertpaibul-Rao's equation[7]:

$$\eta_{rel} = 1 + [\eta]C \tag{5}$$

Higiro's equations[22]:

$$\eta_{rel} = e^{[\eta]C} \tag{6}$$

$$\eta_{rel} = \frac{1}{1-[\eta]C} \tag{7}$$

Estimation of Shape factor and swollen volume parameters. Based on the following equation, intrinsic viscosity depends on two crucial molecular parameters, namely the shape factor and swollen volume[23]:

$$[\eta] = \mathrm{v}.\mathrm{v}_S \quad (8)$$

where, v is the biopolymer shape factor which is known as the viscosity increment, and v_s is the biopolymer swollen volume or the voluminosity. The swollen volume depends on relative viscosity and is determined from the intercept of the plot of Y versus the concentration as follows:

$$Y = \eta_{rel^{0.5}} - 1/[C(1.35\eta_{rel^{0.5}} - 0.1)] \quad (9)$$

Estimation of molecular conformation. The power-law relationship equation (10) was applied to determine the exponent *b*, as the slope of the logarithmic diagram of the specific viscosity versus the concentration. This parameter is generally used to describe the polysaccharide conformation[24].

$$\eta_{sp} = aC^b \quad (10)$$

Determination of the chain flexibility parameter and activation energy. The reduction in the viscosity of the polymer solution in the Newtonian domain with increasing temperature mostly follows the Arrhenius-type equation[12]:

$$[\eta] = Ae^{E_a/RT} \quad (11)$$

where, $[\eta]$ is the intrinsic viscosity (dl/g), A is the model constant, E_a is the activation energy (kJ/kgmol), R is the universal gas constant (kJ/kgmol.K), and T is the absolute temperature (K). Typically, the E_a/R value is an indicator of biopolymer's chain flexibility so that high values of E_a/R indicate low chain flexibility[25].

Statistical analysis. To survey the effect of temperature (at five levels of 10, 25, 40, 55, and 70°C) on the dilute solution properties of NSG, a completely randomized design with three repetitions, and Duncan test at a 95% confidence level were applied for statistical analysis and comparing means by SPSS software version 22, respectively. Also, MATLAB software (R2013a) was used to select the most appropriate model for intrinsic viscosity estimation. Microsoft Excel 2010 software was used to draw the graphs.

Results and discussion

Intrinsic viscosity. Intrinsic viscosity is an indicative of the hydrodynamic volume occupied by each macromolecule[26], which provides an in-depth insight into the molecular characteristics[10]. The interaction between various polymers, the fragments of a polymer chain, and between the polymer chains and the solvent molecules affects the polymer conformation. On the other hand, polymer conformation is affected by temperature and polymer concentration[27]. In this study, the intrinsic viscosity was specified by two methods, that in the first method, it was estimated using the Huggins and Kraemer equations (3), and (4), while in the second method, it was computed by the Tanglertpaibul & Rao equation (5) and Higiro 1 & 2 equations (6), and (7)[11, 20]. Figures 1 and 2 show the results of fitting by the investigated models to the experimental data obtained for NSG in the dilute regime. In the present research, due to the lack of a logical relationship between intrinsic viscosity and temperature changes, Huggins and Kramer's equations were not considered as the appropriate models (Table 1). Slope-based relations (Tanglertpaibul-Rao, Higiro 1 & Higiro 2) showed high efficiency for determination of the intrinsic viscosity of NSG at 25 ^{o}C, because they displayed a better linear fit with maximum R^2 and minimum RMSE values (Table 2). McMillan[20], and Razavi et al.[11], also found that slope-based methods had higher significant correlation coefficients and lower standard errors than those intercept-based methods. It is probably due to the sequential dilution in sample preparation that increases the error in η_{sp}/C term[21]. Besides, Launay[28] reported that the Huggins equation is just credible at η_{sp}< 0.7. In this research, the η_{sp} value of the NSG was between 0.2 and 1.0. Behrouzian et al.[9], Lai et al.[24], and Higiro et al.[29] also applied slope-based methods to estimate the intrinsic viscosity of cress seed gum, Hessian Tsao gum, and xanthan gum, respectively. Lapasin and Prickel[30] stated that for non-ionic polysaccharides (such as acacia bean gum), reduced viscosity (η_{sp}/C) increases with small uniform slope as polymer concentration elevates, and for ionic polysaccharides (such as xanthan), due to the electrostatic repulsion between the chains and the dimensions of the developed helix, reduced viscosity (η_{sp}/C) increases steadily with very rapidly slope with increasing polymer concentration. According to Figure 1, the behavior of NSG is similar to that described for ionic polysaccharides by Lapasin and Prickel[30]. As shown in Table 2, about the effect of temperature, the Higiro 2 model (equation (7)) displayed highest efficiency among the applied models because of the higher R^2 and lower RMSE values obtained. Therefore, this model was selected as the most appropriate model for computing the intrinsic viscosity of the NSG. Based on this model, the intrinsic viscosity value of NSG has obtained as 0.2 dl/gr at 25°C. When the temperature enhanced from 10°C to 70°C, the intrinsic viscosity of NSG decreased from 0.2 to 0.15 dl/gr (Table 2). Because of increasing the vibrations of molecules and intermolecular distance, it may be attributed to the abruption change in the gyration of NSG macromolecules as a result of increasing their chains' flexibility[28,31]. These results were consistent with previous observations[10,12,13,32,33]. Oppositely, Stivala and Bahary[34] expressed that by raising temperature from 25 to 57∘C, the intrinsic viscosity of Levan was increased. Furthermore, Haug and Smidsrod[35] stated that the temperature has little effect on intrinsic viscosity of alginate. In comparison, the intrinsic viscosity of NSG was almost the same as reported for Anghouzeh gum (0.213 dl/gr)[36], and Albizia gum (0.23 dl/gr)[37], and lower than guar (9.25 dl/gr)[38], xanthan gum (214.21 dl/gr)[22], and Nettle seed (*Urtica dioica*) gum (8.56dl/gr)[16], and higher than cashew gum (0.1 dl/gr at 20°C)[39], and gum Arabic (0.177 dl/gr at 25°C)[40].

	Huggins			Kraemer		
Temperature (∘C)	[η]	R^2	RMSE	[η]	R^2	RMSE
10	0.100±0.042^{b}	0.844	0.037	0.168±0.033^{b}	0.689	0.052
25	0.179±0.004^{a}	0.974	0.007	0.217±0.003^{a}	0.920	0.004
40	0.138±0.003^{ab}	0.975	0.005	0.181±0.001^{b}	0.868	0.005
55	-0.395±0.011^{d}	0.872	0.017	-0.249±0.006^{d}	0.781	0.013
70	-0.041±0.016^{c}	0.970	0.006	0.027±0.010^{c}	0.943	0.005

Table 1. Comparison of intrinsic viscosity ([η], dl/gr) of Nettle seed gum (NSG) solution using Huggins and Kraemer models at different temperatures. Results are expressed as means ± SD for three replications. a-d: Means followed by the same letters in the same column for each temperature are not significantly different (P > 0.05).

Temperature (◦C)	Higiro (1)			Higiro (2)			Tanglertpaibul & Rao		
	[η]	R^2	RMSE	[η]	R^2	RMSE	[η]	R^2	RMSE
10	0.269±0.001[a]	0.947	0.057	0.208±0.001[a]	0.986	0.031	0.360±0.001[a]	0.849	0.114
25	0.268±0.001[a]	0.977	0.023	0.208±0.001[a]	0.991	0.009	0.356±0.002[a]	0.899	0.081
40	0.245±0.001[a]	0.959	0.023	0.195±0.001[a]	0.991	0.007	0.315±0.002[ab]	0.868	0.066
55	0.189±0.001[b]	0.813	0.180	0.157±0.001[b]	0.896	0.133	0.233±0.002[bc]	0.718	0.248
70	0.181±0.001[b]	0.792	0.042	0.150±0.000[b]	0.875	0.022	0.222±0.001[c]	0.706	0.072

Table 2. Comparison of intrinsic viscosity ([η], dl/gr) of Nettle seed gum (NSG) solution using Tanglertpaibul & Rao and Higiro models at different temperatures. Results are expressed as means ± SD for three replications. a-c: Means followed by the same letters in the same column for each temperature are not significantly different ($P > 0.05$).

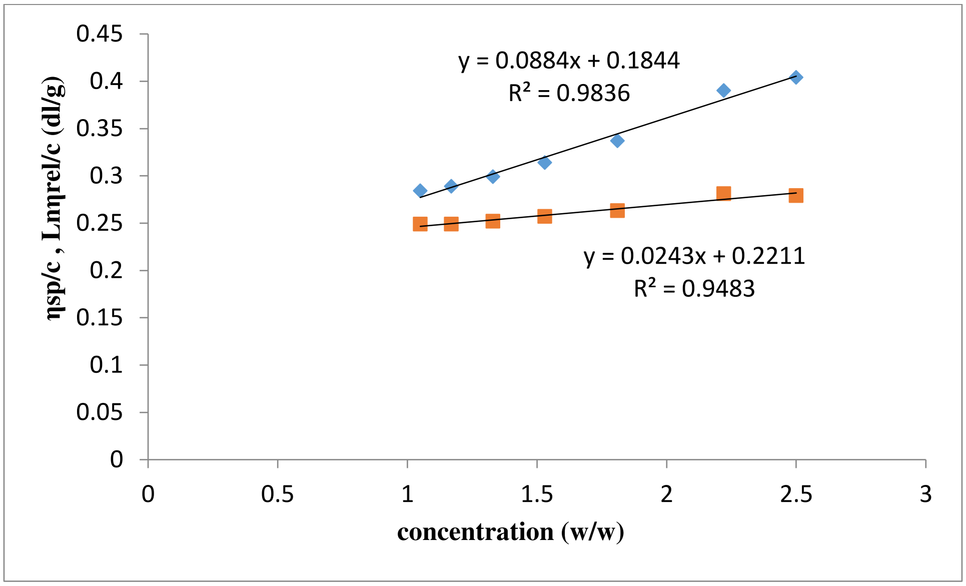

Figure 1. A typical dual Huggins (♦) and Kraemer (■) plots of Nettle seed gum (NSG) in deionized water (25 ◦C).

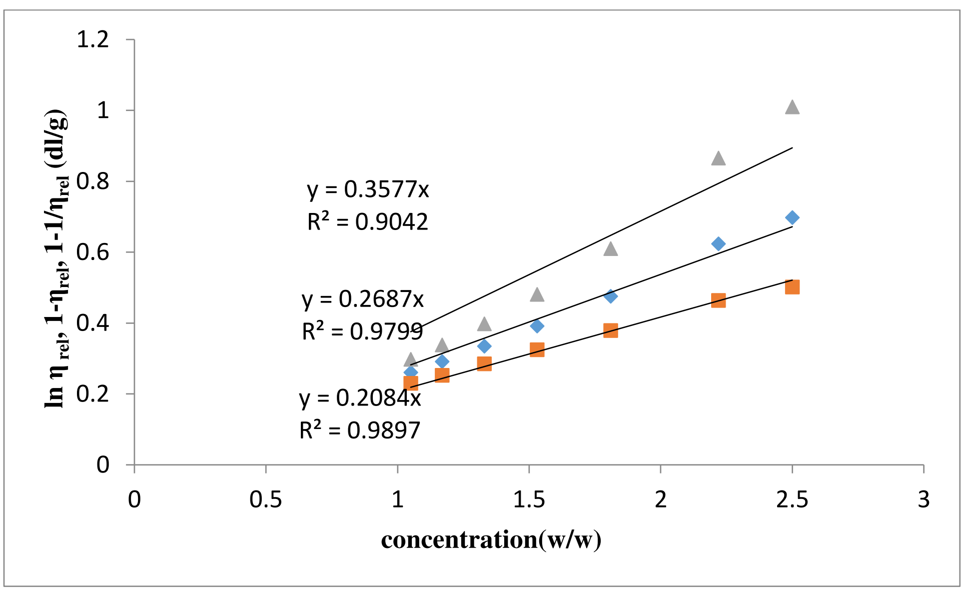

Figure 2. A typical triple Tanglertpaibul-Rao (▲), Higiro 1(♦) & Higiro 2(■) plots of Nettle seed gum (NSG) in deionized water (25 ◦C).

Voluminosity and shape factor. The intrinsic viscosity depends on two critical molecular parameters, namely the shape factor (υ) and voluminosity (v_s). The shape factor is a dimensionless parameter, which indicates the shape of a polymer in solution[41]. If the amount of the shape factor (υ) is equal to 2.5, the macromolecule shape is spherical, higher amount is relevant to the ellipsoidal shape and amounts less than 2.5 show oblate or prolate shapes[23]. As observed in Table 3, at the temperatures below 40 °C, the shape factor value was less than 2.5, indicating that the NSG macromolecules are oblate/prolate in shape. At 40°C, the shape factor was 2.5, so the NSG polymers are spherical in shape. With increasing the temperature from 40°C to 70°C, the shape factor value of NSG increased from 2.55 to 7.24, which represents more expanded conformation (ellipsoidal) at high temperature (Table 3). These findings were consistent with the data of intrinsic viscosity (Table 2). Regarding the impact of temperature on the shape factor value, similar findings were reported by Yousefi et al.[12], and Razavi et al.[11]. Shape factor values of smaller than 2.5 were reported for cress seed gum and dextran gum by Mohammad Amini et al.[10], and Antonio et al.[23], respectively.

Temperature (°C)	v_s (dl/g)	υ
10	0.092±0.019^{a}	2.310±0.418^{c}
25	0.089±0.001^{a}	2.330±0.019^{c}
40	0.076±0.001^{a}	2.550±0.025^{c}
55	0.036±0.002^{b}	4.380±0.272^{b}
70	0.021±0.004^{b}	7.240±1.119^{a}

Table 3. Voluminosity (v_s) and shape factor (υ) of Nettle seed gum (NSG) at different temperatures. Results are expressed as means ± SD for three replications. a-c: Means followed by the same letters in the same column for each temperature are not significantly different ($P > 0.05$).

The voluminosity or the swollen specific volume (v_s) represents the polymer configuration in various solvent conditions[11,23]. Solvent bonding causes the anhydrous macromolecule to expand when dissolved or dispersed in water so that v_s can be supposed as a scale of solvent bonding with macromolecule or the macromolecule volume in solution per unit mass of biopolymer without water[42]. The values of voluminosity (v_s) of NSG at different temperatures are shown in Table 3. By elevating the temperature from 10°C to 70°C, the swollen specific volume declined that shows the NSG polymer dimensions or the solvent power decreased[11]. As voluminosity is described as the volume of the hydrated biopolymer per unit mass of dry biopolymer, the achieved results from v_s validated the obtained findings of the intrinsic viscosity (Table 3). Similar findings regarding the impact of temperature on v_s were reported by Razavi et al.[11].

The molecular conformation. The coil overlap parameters including the master curve slope (MCS), critical concentration (C^*) and the berry number ($C[\eta]$). The plot of log (η_{sp}) versus log $C[\eta]$, which is known as the ''master curve'', is applied to designate the coil overlap parameters in the dilute regime[43,44]. The berry number is a dimensionless parameter representing the volume taken up by the polymer molecules in solution[43,44]. The molecular entanglements occur when Berry number ($C[\eta]$) is higher than one[45,46], so in semi-dilute solutions, the Berry number is in the range of 1.0-10.0[45]. In the present research, this parameter calculated for NSG solution was in the range of 0.21-0.52 at 25°C, which indicates that no coil overlap and molecular entanglements happened (Table 4).

Temperature (◦C)	MCS	C[η]	*B*
10	0.76±0.00	0.29-0.51	1.42±0.40^{b}
25	0.81±0.00	0.21-0.52	1.44±0.01^{b}
40	1.12±0.00	0.25-0.48	1.56±0.01^{b}
55	1.32±0.40	0.21-0.39	1.95±0.04^{a}
70	1.40±0.00	0.24-0.37	2.21±0.07^{a}

Table 4. The values of master curve slope (MCS), Berry number (C[η]) and exponent *b* (slope of log η_{sp} vs. log C) of Nettle seed gum (NSG) solution at different temperatures. Results are expressed as means ± SD for three replications. a-b: Means followed by the same letters in the same column for each temperature are not significantly different ($P > 0.05$).

In a dilute solution, the master curve slope (MCS) value is less than 1.4, whereas for semi dilute regime, the MCS has been found in some cases close to 3.75, However, the published values of the slopes may vary for random coil polymers in good solvent from 3.4 up to 5[43]. At the temperature range of 10-70°C, the master curve slop for the NSG solution was less than 1.4, indicating that it was within the dilute solution domain and that no molecular entanglements was present (Table 4).

The parameter *b*, the slope of the power-law model (equation (10)), is displayed in Table 4. Morris et al.[44] reported that within the dilute domain, parameter *b* values higher than the number one is relevant to random coil conformation. In contrast, fewer values are a demonstration of rod-like conformation. The parameter *b* values for NSG at selected temperatures were ranged from 1.42 to 2.2, confirming the random coil conformation of the NSG macromolecules. Similar data were reported by Yousefi et al.[12] for sage seed gum. They also found that by elevating the temperature from 25 to 65 °C, the amount of parameter *b* increases.

Chain flexibility parameter and activation energy. The reduction in the viscosity of the polymer solution in the Newtonian domain with increasing the temperature generally follows the Arrhenius law. If the intrinsic viscosity was used in place of the Newtonian viscosity, the slope of the logarithmic plot of the intrinsic viscosity ($\ln[\eta]$) against the inverse of absolute temperature ($1/T$) is applied as an indicator of macromolecular chain flexibility (E_a/R)[47]. Thus, high E_a/R value indicates low chain flexibility[25]. The chain flexibility parameter (E_a/R) and activation energy (E_a) calculated for the NSG at the studied temperatures were 587.2 K and 0.488×10^7 J/kgmol, respectively. The chain flexibility parameter of NSG was lower than the value obtained for sage seed gum (3046.45)[12], Balangu seed gum (1156.53)[10], xanthan (1100)[48], cellulose diacetate (645)[49], Qodumeh Shirazi seed gum (618.54)[15], but it was more than chitosan (488)[50], indicating greater flexibility of the macromolecular chain of NSG in comparison to most hydrocolloids. Also, the value for the activation energy (E_a) of NSG was lower than sage seed gum (2.53×10^7 J/kgmol)[12], chitosan (2.5×10^7 J/kgmol)[51], Balangu seed gum (1.00×10^7 J/kgmol)[10], and almost similar to Qodumeh Shirazi seed gum (0.51×10^7 J/kgmol)[15].

Conclusion

In this paper, the influence of temperature (10, 25, 40, 55, and 70◦C) on some molecular parameters and intrinsic viscosity of NSG in the dilute regime has been examined. After fitting different models, the Higiro 2 model was selected as the best model to explain the behavior of the dilute solution of NSG. According to the results, with increasing the temperature, the amount of intrinsic viscosity, and voluminosity (v_s) decreased. The results of the shape factor (v) showed that at temperatures below 40°C, the NSG macromolecules are oblate/prolate in shape but at 40°C, they are spherical. With raising the temperature from 10°C to 70°C, the shape of NSG changed to ellipsoidal, indicating more expanded conformation at that high temperatures. Berry number and master curve slope (MCS) revealed that NSG solution at all temperatures was within the range of dilution solution regime and that no molecular entanglements were present. The value of the *b* parameter at different temperatures was acquired in the range of 1.4-2.2, which indicates the NSG conformation was the random coil. Based on the Arrhenius-type model parameters, which expresses the chain flexibility parameter of the macromolecules, it was shown that NSG has a relatively flexible chain.

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Author contributions

Z.Z. done the experiments, analyzed and interpenetrated the data, prepared the manuscript and revised the paper. S.M.A.R. designed the research, provided the fund and laboratory facilities, checked the data, controlled the analysis, edited the paper, revised the paper and submitted the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Figures

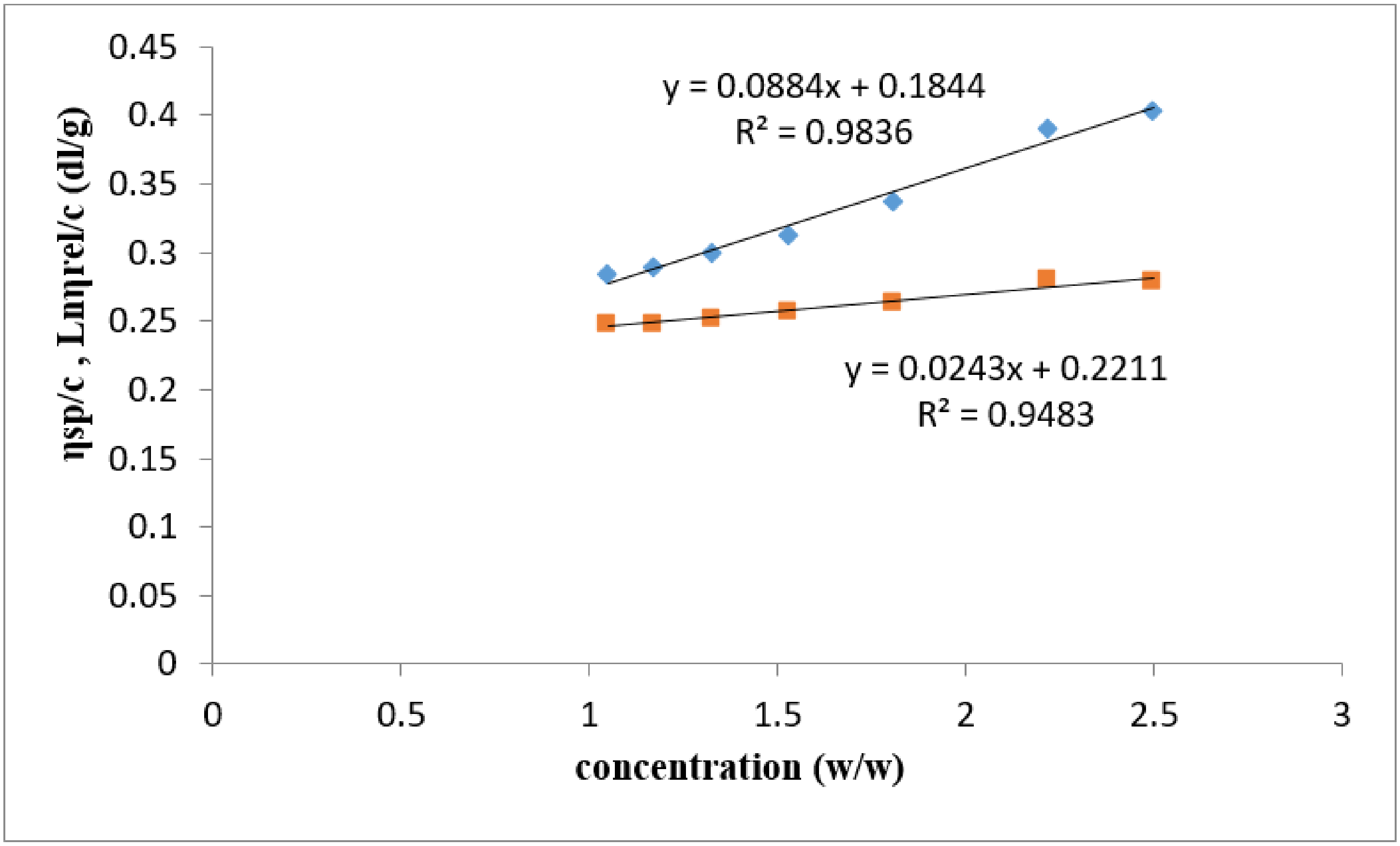

Figure 1

A typical dual Huggins (◆) and Kraemer (■) plots of Nettle seed gum (NSG) in deionized water (25 ℃).

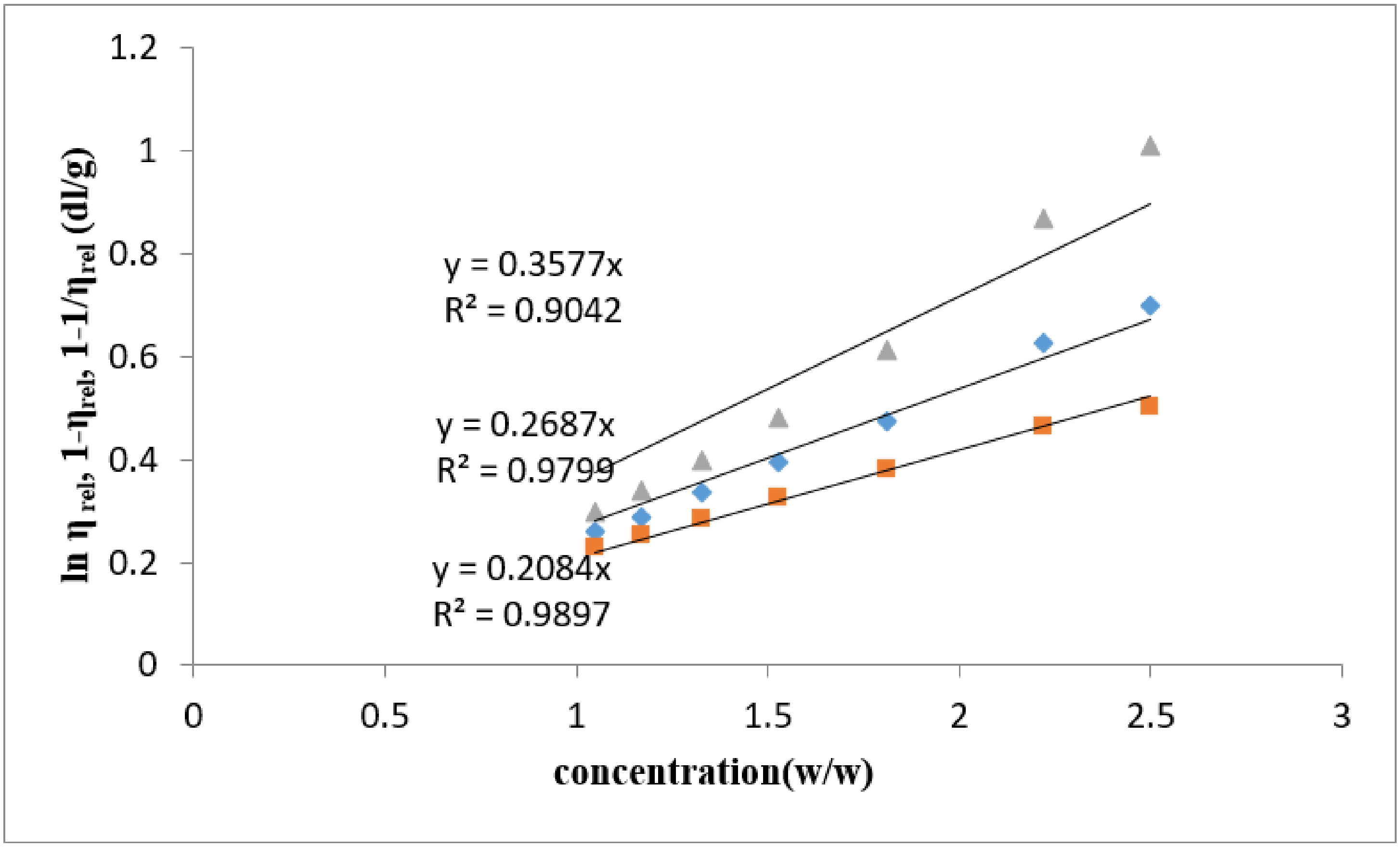

Figure 2

A typical triple Tanglertpaibul-Rao (), Higiro 1() & Higiro 2() plots of Nettle seed gum (NSG) in deionized water (25 C).