Rheology of Zein Solutions in Aqueous Ethanol†

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Aqueous ethanol solutions prepared with commercial zein exhibited Newtonian behavior. Temperature, zein concentration, and ratio of water to ethanol affected viscosity of the zein solutions. The influence of temperature on zein solution viscosity was expressed by an Arrhenius-type equation. As zein concentration increased, solution viscosity exponentially increased. Generally speaking, viscosity decreased when the ethanol concentration increased. Parameters were estimated for an Arrhenius-type equation to describe the viscosity as a function of temperature, zein concentration, and ethanol concentration.

Keywords: *Zein; aqueous ethanol; concentration; temperature; viscosity*

INTRODUCTION

Zein is a prolamin fraction of corn protein. It has unique solubility characteristics due to its strongly hydrophobic nature and can be dissolved in aqueous polar organic solvents such as aqueous alcohol solutions (Manley and Evans, 1943; Fu, 1999). Therefore, zein can be easily separated from other water-soluble protein and starch by suspension in an aqueous alcohol solvent (Swallen, 1941). Gluten meal is a traditional byproduct of the corn wet-milling industry. It contains 47% (db) of zein proteins and is the main source of commercial zein and laboratory zein. Zein has been shown to have adhesive properties and to be able to form films and fibers (Pomes, 1971). Films produced using zein have excellent water barrier properties because of zein's hydrophobic character and stability in acid and alkaline solutions (Gennadios et al., 1994). Therefore, zein has been employed as a raw material for waterproofing paper, coating wood, adhering plywood, damp-proofing, and coating foods and tablets (Reiners et al., 1973; Takahashi, 1995).

Viscosity is a major physical property for zein solutions. It is an important parameter to be considered in process design as it determines agitation and pumping power requirements in processes such as extraction and purification. Observation of changes in viscosity may lead to a better understanding of the conformational changes in zein in different ratios of ethanol−water solvents. A simple model of zein solutions would be very helpful in engineering process design.

Most fluid foods exhibit rheological behaviors that follow a power law (Rao et al., 1984; Steffe, 1992):

$$\tau = K(\dot{\gamma})^n \quad (1)$$

When $n = 1$, the fluid is known as a Newtonian fluid. Many fluid foods, such as fruit juice, milk, honey, and light vegetable oil, are Newtonian fluids, in which shear stress is directly proportional to shear rate (Steffe, 1992):

$$\tau = \eta(\dot{\gamma}) \quad (2)$$

Several experimental models are available for the description of rheological behaviors of fruit juices according to different temperatures and concentrations (Vitall and Rao, 1984; Khalil et al., 1989).

However, limited work has been reported for protein solutions, especially for mixtures of water and organic solvents. Therefore, the object of this research was not only to fit a model to predict the viscosity of zein solutions at various conditions, such as zein concentration, ethanol concentration, and temperature, but also to provide insight into physical and chemical changes that may occur with the zein under different conditions.

MATERIALS AND METHODS

Reagents. Commercial zein (regular grade, F-4000) was obtained from Freeman Industries Inc., Tuckahoe, NY. This sample was characterized as α-zein (Wilson, 1988; Matsumura et al., 1997) and contained on a wt/wt dry basis 14.89% nitrogen, 1.03% ash, and 1.18% crude fat (Fu, 1999). The zein sample contained 7.5% (wet basis) moisture. Dehydrated ethanol (100%) was obtained from McCormick Distilling Co., Inc., Weston, MO.

Sample Preparation. Zein solutions were prepared by mixing the proper amount of zein in aqueous ethanol [50, 60, 70, 80, 85, or 90% (wt of ethanol/wt of ethanol and water)] to obtain 2, 4, 6, 8, 10, 12, or 14% (wt of zein/wt of total) zein for rheological measurements. The order of sample preparation for combining the various zein and ethanol concentrations was randomly decided. Zein sample weight was adjusted to account for its initial moisture content [≈7.5% MC (wb)] by converting to a dry basis, and ethanol concentration also was adjusted to account for the extra water added in the zein sample to adjust the ethanol and zein concentration as desired. Viscosity measurements were made within 15 min of sample solution preparation.

Viscosity Measurement. Viscosity of each zein solution was measured at least twice for each sample using a cone viscometer (Bookfield Digital Viscometer, model LVT/PDV-II+) equipped with a CP-51 cone spindle (angle = 1.565° and radius

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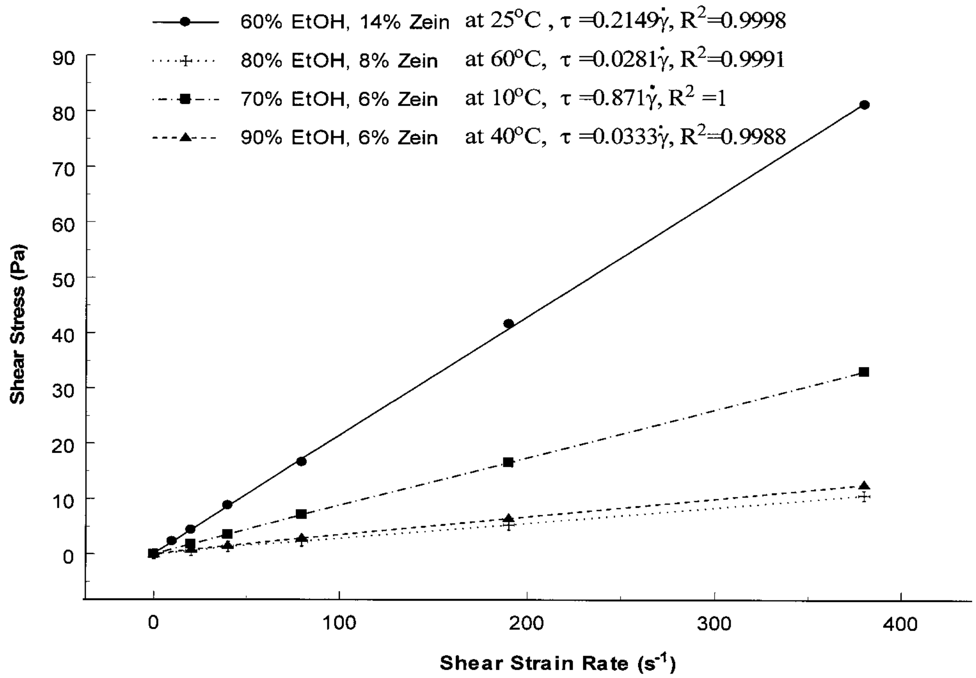

Figure 1. Example of zein solution rheograms at various combinations of zein concentration, ethanol concentration, and temperature.

= 1.2 cm). A thermostatic bath was used to control the working temperature (10, 20, 30, 40, 50, or 60 °C) of each solution within the viscometer. A 0.5 mL sample was taken for each measurement. Viscosity and shear stress readings were recorded 30 s after spindle rotation had been initiated. Viscosity measurement of samples with aqueous ethanol concentration of 50% and zein concentrations of ≥10% were observed and recorded between 20 and 60 °C due to the limited solubilities of zein at temperatures <10 °C. Viscosity was measured within 15 min after preparation of the zein solutions, as it is known to change with time under conditions of quiescent storage and room temperature (Evans and Manley, 1943; Fu, 1999).

The shear strain rate ($\dot{\gamma}$) was calculated from the speed setting and cone geometric dimensions using the manufacturer's recommendations. Shear stress (τ) and instantaneous viscosity (η) values were read directly from the digital display on the viscometer. Preliminary tests indicated varying temperature, ethanol concentration, and zein concentration all influenced the viscosity; however, the zein solutions still exhibited Newtonian behavior. Therefore, the viscosity (η) for all zein solutions was recorded at a spindle speed of 100 rpm because a previous study found the readings at 100 rpm were more stable than those at lower spindle speeds (D. Fu, unpublished results, 1997).

Parameter Estimation. An optimization program in a statistical package was employed to determine parameters in a combined model with ethanol concentration, zein concentration, and temperature. The parameters were estimated for the nonlinear regression model through PROC NLIN (release 6.12 for Windows, SAS Institute Inc., Cary, NC) according to the least-squares mean method of Marquardt (1963).

RESULTS AND DISCUSSION

Rheological Behavior. The Newton model was applied to describe the rheological behavior for the zein solutions. Figure 1 shows an example of the relationship between shear stress and shear stain rate for several zein concentrations over a range of aqueous ethanol ratios and at various temperatures. Similar rheograms were obtained for the other concentrations of zein in various solutions of aqueous ethanol at different temperatures. However, these additional rheograms were not included in Figure 1 due to space limitations.

Zein solutions were characterized as Newtonian fluids on the basis of their constant viscosity within any combination of zein concentration, ethanol concentration, and temperature. For any zein solution, viscosity did not change as the shear strain rate changed for a fixed temperature, zein concentration, and ethanol concentration. However, the solutions were not stable. The zein tended to form a gel that set spontaneously over time to a solid gel (Swallen, 1941; Evans and Manley, 1941, 1943; Fu, 1999).

Effect of Temperature. The effect of temperature on viscosity for Newtonian fluids can be expressed by an Arrhenius-type equation (Khalil et al., 1989):

$$\eta = \eta_0 \exp(E_a/R_gT) \qquad (3)$$

To solve for the two parameters of η_0 and E_a, eq 3 may be written in logarithmic form as a simple linear relationship

$$\ln \eta = A + B(1/T) \qquad (4)$$

where $A = \ln \eta_0$ and $B = E_a/R_g$. The coefficients of determination (R^2) calculated for the data were >0.97 (Table 1) ($p < 0.05$) for the solutions of various zein and ethanol concentrations between 10 and 60 °C using eq 4. Parameters (E_a and $\ln \eta_0$ in Table 1) for the various zein concentrations in different ethanol concentrations were estimated using the viscosity at the corresponding temperatures of 10, 20, 30, 40, 50, and 60 °C, respectively. Generally speaking, the activation energy decreased with increasing ethanol concentration. Danzer et al. (1975) observed that changes in ethanol concentration could alter the conformation of zein in various ratios of aqueous ethanol solvents. Therefore, activation energy change with ethanol concentration change is likey related to protein conformational change.

Effect of Zein Concentration. The viscosity exponentially increased with increasing solid concentration in many cases (Rao et al., 1984; Steffe, 1992). Similar results were observed for various zein concentrations

Table 1. Frequency Factor and Activation Energy for Various Zein Concentrations and Ethanol Concentrations between 10 and 60 °C[a]

zein concn (%)	ethanol concn (%)	$\ln \eta_0$	E_a (kJ/mol)	R^2
2	50	7.23	21.12	0.996
	60	7.01	20.45	0.990
	70	6.21	18.22	0.987
	80	5.93	17.24	0.990
	90	5.18	15.01	0.971
4	50	7.19	21.77	0.998
	60	6.62	20.22	0.998
	70	5.79	17.82	0.986
	80	5.46	16.74	0.995
	90	4.67	14.41	0.994
6	50	6.89	21.73	0.993
	60	6.25	20.02	0.994
	70	6.17	19.60	0.991
	80	5.78	18.27	0.995
	90	4.89	15.84	0.995
8	50	6.85	22.48	0.994
	60	7.11	22.93	0.991
	70	7.00	22.38	0.990
	80	6.54	21.02	0.982
	90	4.75	16.27	0.985
10	50	6.14	21.36	0.997
	60	6.47	22.00	0.987
	70	6.04	20.77	0.990
	80	6.48	21.62	0.998
	90	4.48	16.53	0.984
12	60	6.14	22.10	0.999
	70	5.17	19.45	0.995
	80	5.50	20.09	0.997
	85[b]	5.27	19.45	0.993
	90	4.22	16.94	0.995

[a] Each line corresponding to a zein and ethanol concentration combination was determined using six temperature points with each temperature point duplicated. [b] Because of solubility limitations at 12% zein in 50% ethanol, 85% ethanol was substituted.

Table 2. Linear Relationship Coefficients Determined Using ln η and Zein Concentration for Various Temperatures and Ethanol Concentrations[a]

ethanol concn (%) (w/w)	temp (°C)	$\ln \eta = A_1 + B_1 C_{zein}$		
		A_1	B_1	R^2
50*	10*	1.43	16.14	0.997
	20	1.09	15.59	0.997
	30	0.87	14.09	0.999
	40	0.54	15.40	0.999
	50	0.34	15.09	0.998
	60	0.11	14.43	0.999
60	10	1.37	15.85	0.997
	20	1.10	14.12	0.996
	30	0.80	14.80	0.994
	40	0.47	15.23	0.993
	50	0.32	14.35	0.993
	60	0.12	13.90	0.998
70	10	1.17	16.72	0.994
	20	0.98	14.26	0.983
	30	0.71	14.81	0.998
	40	0.41	15.20	0.989
	50	0.30	14.09	0.982
	60	0.03	14.82	0.991
80	10	1.04	16.92	0.990
	20	0.77	15.38	0.996
	30	0.59	15.14	0.998
	40	0.34	15.16	0.995
	50	0.15	14.88	0.989
	60	0.02	14.14	0.996
90	10	0.84	17.80	0.993
	20	0.56	16.98	0.998
	30	0.37	16.86	0.996
	40	0.21	16.59	0.992
	50	0.05	16.35	0.996
	60	−0.11	16.51	0.995

[a] Each line corresponding to an ethanol concentration and temperature combination was determined using six points of zein concentrations except as indicated by an asterisk, which used only five points due to solubility limitations for 12% zein.

in aqueous ethanol solutions at various temperatures (Table 2). For any given temperature and ethanol concentration combination, the results indicated that ln η linearly increased with zein concentration ($R^2 > 0.98$ at $p < 0.05$). Table 2 lists estimated parameters of A_1 and B_1 for eq 5 for ln η versus zein concentration in various ethanol concentrations and at different temperatures.

$$\ln \eta = A_1 + B_1 C_{zein} \tag{5}$$

From Table 2, the slopes (B_1) ranged between 13.9 and 17.8. The mean of all the slopes was found to be 15.38 with a standard deviation of 1.06. All of the data lines are nearly parallel based on the B_1 value (slope) in Table 2. The intercepts (A_1), however, increased with increasing temperature.

Effect of Ethanol Concentration. The experimental results indicated that the zein viscosity was affected by the ratio of ethanol to water (ethanol concentration) (Figure 2). A linear relationship with ln η decreasing as the ethanol concentration (moles per mole) was increasing could be observed at low temperature (10 °C). Quadratic relationships were developed between ln η and ethanol concentration (moles per mole) with increasing temperature. The minimum value for viscosity of the zein solutions was obtained at ~80% ethanol (w/w) at temperatures >30 °C.

Ethanol concentration may influence polymerization of zein molecules in solution as viscosity is directly related to molecular size. The viscosity change due to an ethanol concentration increase suggested that zein molecules were not single units uniformly distributed in the aqueous ethanol. Polymorphism has been detected by gel filtration of zein solutions and small-angle X-ray scattering (Landry et al., 1967; Mastsushima et al., 1997). Zein would appear to be less polymerized in an 80% ethanol as evidenced by the lower viscosity. This observation agrees with zein solubility results. Zein molecules with a lower degree of polymerization and lower molecular weight have greater solubility. The maximum solubility was observed at ~70−80% (w/w) of ethanol concentration (Mossé, 1960; Fu, 1999).

Model Building. B_1 can be assumed to be a constant for eq 5 because B_1 had little variation (standard deviation was 1.06) at various temperatures and ethanol concentrations (Table 2). However, A_1 was a function of temperature and ethanol concentration.

Figure 3 with A_1 versus $1/T$ for various ethanol concentrations demonstrates that a linear relationship ($R^2 > 0.98$) between A_1 and $1/T$ existed where

$$A_1 = A_2 + B_2(1/T) \tag{6}$$

Linear relationships were also observed between A_2 and the ethanol concentration and B_2 and ethanol concentration by plotting A_2 versus ethanol concentration and B_2 versus ethanol concentration (Figure 4). Thus, the following two equations can be used for developing the

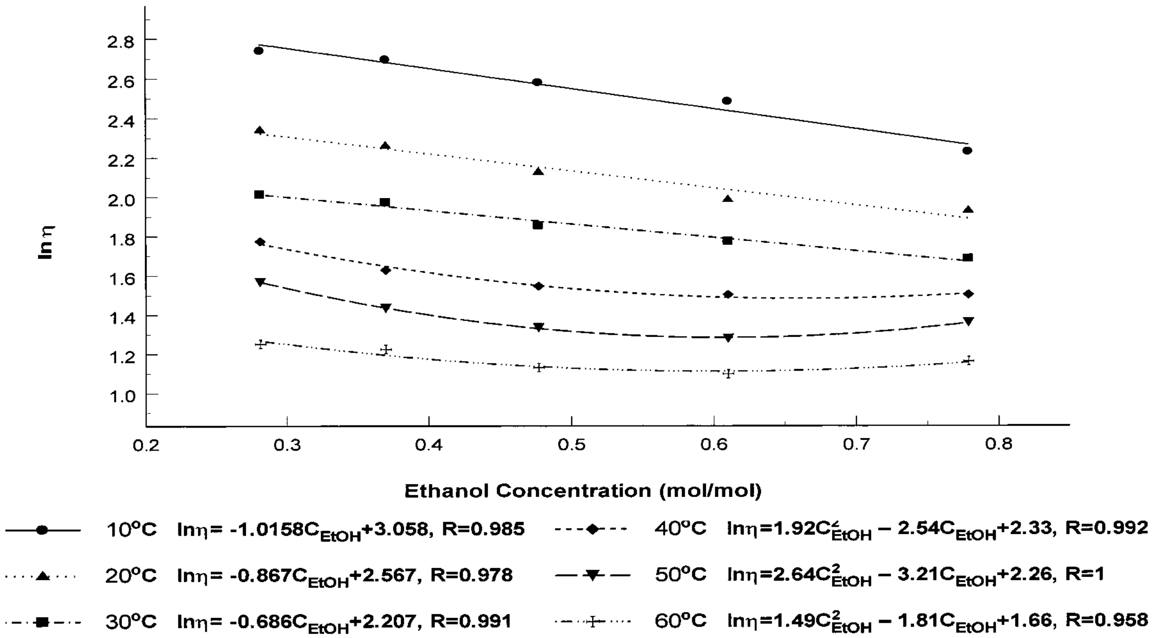

Figure 2. ln η vs C_{EtOH} (moles of ethanol per mole of ethanol and water) in 8% (w/w) zein concentration at various temperatures.

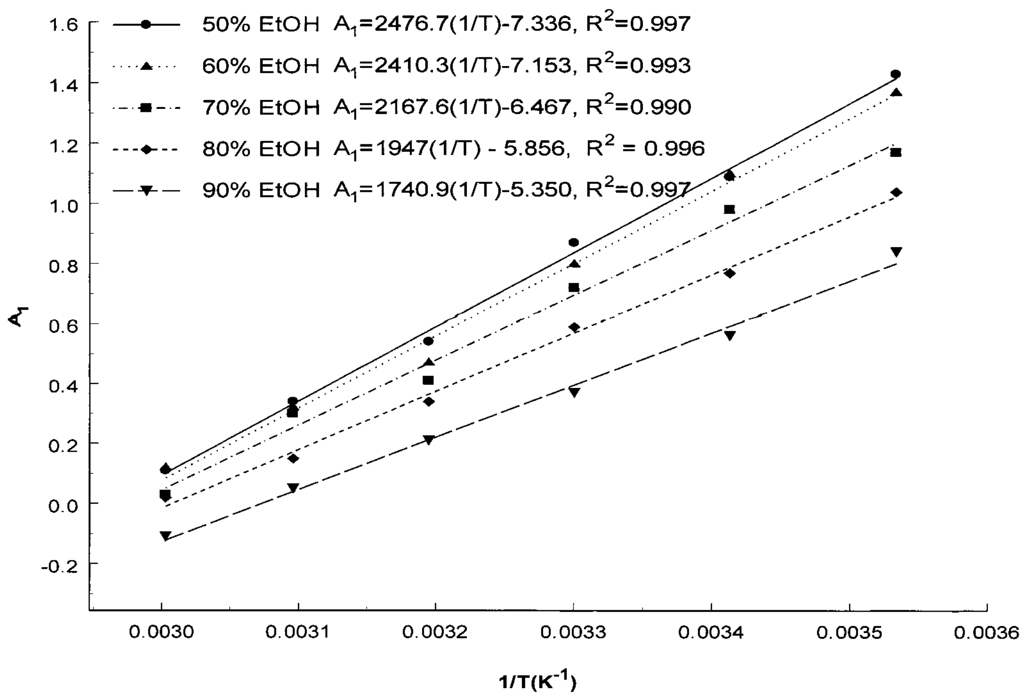

Figure 3. Parameter A_1 in eq 5 vs temperature in different ethanol concentrations (C_{EtOH}).

empirical relationships:

$$A_2 = A_3 + B_3 C_{\mathrm{EtOH}} \quad (7)$$

$$B_2 = A_4 + B_4 C_{\mathrm{EtOH}} \quad (8)$$

Combining eqs 5−8, a viscosity model can be expressed as

$$\ln \eta = K_1 C_{\mathrm{zein}} + (K_2 C_{\mathrm{EtOH}} + K_3)/T + K_4 C_{\mathrm{EtOH}} + K_5 \quad (9)$$

or rewritten as

$$\eta = [k_0 \exp(k_1 C_{\mathrm{zein}} + k_4 C_{\mathrm{EtOH}})] \times [\exp(k_2 C_{\mathrm{EtOH}} + k_3)/R_g T)] \quad (10)$$

that is

$$\eta = \eta_0 \exp(E_a/R_g T) \quad (3)$$

where

$$\eta_0 = k_0 \exp(k_1 C_{\mathrm{zein}} + k_4 C_{\mathrm{EtOH}}) \quad (11)$$

$$E_a = k_2 C_{\mathrm{EtOH}} + k_3 \quad (12)$$

and k_0, k_1, k_2, k_3, and k_4 are constant parameters.

To determine the parameters, viscosity values were fitted to the above multiple nonlinear regression model using least squares. The parameters were estimated using Marquardt's (1963) method run through PROC

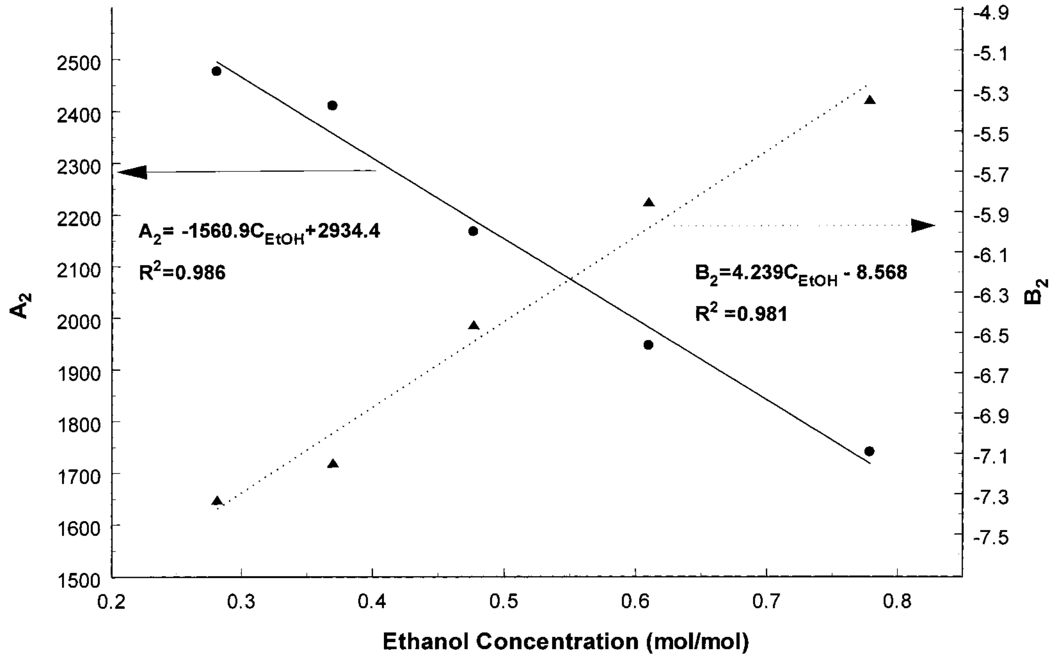

Figure 4. Parameters A_2 and B_2 in eq 6 vs ethanol concentration.

Table 3. Parameters Estimated (R^2 = 0.99) Using PROC NLIN of SAS for Predicting Viscosity Based on Zein Concentration, Ethanol Concentration, and Temperature of Aqueous Ethanol Solutions with Zein

exponential model:[a]
$\eta = k_0 \exp(k_2C_{zein} + k_4C_{EtOH}) \exp[(k_3C_{EtOH} + k_5)/RT]$[b]

parameter	value	units
k_0	1.02×10^{-4}	Pa·s
k_2	15.53	
k_3	−12.38	kJ/mol
k_4	4.23	
k_5	25.69	kJ/mol

[a] Units: viscosity in Pa·s; temperature in Kelvin; zein concentration in weight fraction for the total solution; ethanol concentration in mol fraction for aqueous ethanol system. [b] The model can be rewritten as $\eta = \eta_0 \exp(E_a/RT)$, where $\eta_0 = k_0 \exp(k_2C_{zein} + k_4C_{EtOH})$ and $E_a = k_3C_{EtOH} + k_5$.

NLIN (Table 3). The R^2 reached 0.99 for the model fitted with the viscosity values.

From the viscosity model, activation energies of zein viscosity indicated a linear function dependence on ethanol concentrations. An exponential relationship existed between frequency factors and zein and ethanol concentrations.

Implications. By changing the form of eq 10, it can be rewritten as follows:

$$C_{zein} = [\ln \eta - k_4C_{EtOH} - (k_2C_{EtOH} + k_3)/R_gT - \ln k_0]/k_1 \quad (13)$$

From eq 13, zein concentration can be calculated or predicted using the measured viscosity of a zein solution at a known ethanol concentration and temperature.

At present, Kjeldahl's method and spectrometry methods using protein color reactions are common methods for determining protein concentration. Zein concentration also can be measured by using these methods. Determining the zein concentration by measuring the viscosity may one day provide a simple, quick method. Such a method is largely physical in nature, requiring no chemical reactions and limiting environmental concerns. Further research is needed to determine the influence of zein recovery and purity on a prediction method of this type.

ABBREVIATIONS USED

A, B, constants in eq 4 ($A = \ln \eta_0$ and $B = E_a/R_g$); A_1, B_1, constants in eq 5 for a fixed temperature and ethanol concentration; A_2, B_2, constants in eq 6 for an ethanol concentration; A_3, B_3, constants in eq 7; A_4, B_4, constants in eq 8; K_i (i = 1−5), parameter in eq 9; k_i (i = 0−4), parameter in eq 10; C_{EtOH}, fraction of the ethanol in aqueous ethanol (moles of ethanol per mole of ethanol and water); C_{zein}, zein concentration in aqueous ethanol solution (weight of zein per weight of total); K, consistency index (Pa·s^n); n, flow behavior index; R_g, universal gas constant (0.008314 kJ/mol); R, correlation coefficient; R^2, coefficient of determination; T, absolute temperature (K); η, viscosity (Pa·s); η_0, frequency factor (Pa·s); τ, shear stress (Pa); $\dot{\gamma}$, shear strain rate (s^{-1}).

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