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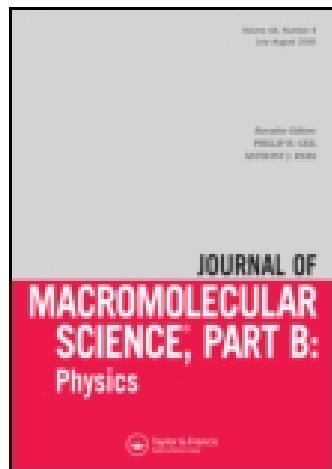
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### Viscosity of Collagen Solutions: Influence of Concentration, Temperature, Adsorption, and Role of Intermolecular Interactions

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# Viscosity of Collagen Solutions: Influence of Concentration, Temperature, Adsorption, and Role of Intermolecular Interactions

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*An investigation of the influences of the adsorption, electrolyte, shear rate, and temperature on the viscometric behavior of collagen solutions was performed using a photoelectric viscometer. The experimental results showed an abnormal behavior of the reduced viscosity ( $\eta_{sp}/C$ ) of dilute collagen solutions measured by a viscometer with or without a hydrophobic surface treatment; the reduced viscosity increased with decreasing concentration. This phenomenon can be completely eliminated by increasing the concentration of an added salt. It indicated that the abnormal viscosity resulted from a long-ranged, inter-molecular electrostatic force. The reduced viscosity decreased as the shear rate increased. The shear-thinning cavitation phenomenon resulted from the high aspect ratio of the collagen molecules. The reduced viscosity of the collagen solutions increased with an increase in temperature, which was due to the association of collagen molecules at high temperature.*

**Keywords** adsorption, collagen solution, electrostatic force, shear rate, viscosity

## Introduction

Collagen is an extracellular protein responsible for most of the mass and strength of structural tissues such as bone, tendon, skin, and cartilage. The collagen molecule is characterized by a triple helical structure and is the structural element of the fibrils of various types forming the collagen family.<sup>[1,2]</sup> It is well documented that the most abundant, type I collagen, is comprised of two identical  $\alpha 1(I)$  chains and one  $\alpha 2(I)$  chain. Each of them has the repeating triplet amino acid sequence of Gly-X-Y, in which X and Y are frequently proline (Pro) and hydroxyproline (Hyp), respectively.<sup>[3]</sup> The nature of the triple helical conformation had been elucidated initially through X-ray diffraction and later confirmed by X-ray crystallographic and computational studies of collagen like peptides.<sup>[4–7]</sup> The collagen fibrils consist of triple helices (1.5 nm in diameter) with a long helical region, about 300 nm in length, and short nonhelical N and C-terminal telopeptides.<sup>[8]</sup>

It is well known that viscometry is a simple and effective method to investigate the conformational change of macromolecules in solution. In case of flexible polyelectrolytes, a decrease of the ionic strength may lead to an expansion of the coils and an increase of

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solution viscosity, which is due to strong intermolecular forces. The curves of reduced viscosity vs. concentration of flexible polyelectrolytes upward bend, indicative of abnormalities of the viscosity behavior. For instance, Gisela et al. synthesized rodlike poly(p-phenylene) (PPP) polyelectrolytes, and found an increase of the reduced viscosity upon dilution in aqueous solutions.<sup>[9]</sup> Song studied the viscosities of aqueous solutions of the semirigid cellulose at different temperatures, from dilute to extremely dilute concentrations, and found that the curve of reduced viscosity plotted against concentration showed an upward trend with decreasing concentration.<sup>[10]</sup> Miyazaki, through simulation, found that for the viscosity of rodlike polyelectrolyte solutions there was a peak at the overlap threshold concentration, and the peak height decreased with increased concentrations of added salt.<sup>[11,12]</sup> However, abnormalities of viscosity behaviors of rigid rodlike biomacromolecule solutions has rarely been reported experimentally in the literature.

Gelatin is a hydrolyzed form of collagen. Domenek et al. found an increase of the reduced viscosity upon dilution of aqueous gelatin solutions.<sup>[13]</sup> Collagen is a typical rigid rodlike biomacromolecule. Although much attention has been paid to studies of the properties the collagen solutions,<sup>[14]</sup> few efforts have been made to study the viscosity of collagen dilute solutions. Since collagen molecules are prone to aggregate in solutions with high concentration, the single macromolecular conformation can most readily be investigated in dilute solution. Kahn et al. studied the viscometric behavior of a solubilized collagen at low rates of shear and found that, under Newtonian conditions, the intrinsic viscosity of collagen solution as expected, decreased with an increase in temperature.<sup>[15]</sup> On the other hand, Ronzon et al. carried out the characterization of acid-soluble type I collagen in solution by viscosity measurements, and found the intrinsic viscosity values were larger at 25°C than that at 4°C.<sup>[16]</sup> However, systematic studies on the viscometric behavior of collagen are absent. Abnormalities of viscosity behavior, if any, have not been described yet.

There are several reasons for the above inconsistent results on the effect of temperature on collagen solution viscosity. First, the sources of the collagen were diverse and its purity may be different even for the same source with different extraction methods. Second, the phenomenon of adsorption will be unavoidable for capillary surfaces without hydrophobic treatment, and the precision of the instruments were relatively low. In this work, the viscosities of dilute collagen solutions were carefully measured at various concentrations, temperatures, ionic strength, and shear rate via a horizontal low shear photoelectric viscometer with high precision. It was found that abnormalities of viscosity behavior with concentration existed in dilute collagen solutions. The effect of adsorption, electrolyte, shear rate, and temperature were measured.

## Experimental

### Materials

The collagen sample (type I, from calf skin) and acetic acid were purchased from Sigma-Aldrich Co. LLC., USA. The collagen solutions, ranging in concentration from  $10^{-3}$  to  $10^{-5}$  g/cm<sup>3</sup> were prepared as follows. The collagen was dissolved in 0.1 M acetic acid solutions at room temperature and stirred for 2 hr. Then the mixture was centrifuged at 3000 r.p.m. at 4°C for 1 h and the supernatant fluid was taken for the viscosity measurements. The pH of the collagen solutions was approximately 2.76.

### Viscosity Measurements

A gravitational capillary viscometer with a horizontal loop and a capillary radius of around 0.04 cm was used to obtain the viscosities for all of the as-prepared, diluted collagen solutions; it was placed in a thermostatically controlled bath. The data analysis was based on the rheometric theory reported by Ye et al.<sup>[17]</sup> The temperature was measured near the capillary by a thermometer with an accuracy of  $\pm 0.01$  K. The constancy of the solvent flow time served as a criterion for judging the cleanliness of the viscometer and the consistency of the experimental conditions.

A 10 ml of the solvent (0.1 M acetic acid aqueous solution) was first transferred into a thoroughly cleaned viscometer (cleaned with a chromic acid mixture), and its flow time was measured, namely  $t_0$ . After cleaning and drying the viscometer completely, a definite volume of a stock collagen solution with a known weight concentration was added to the viscometer; equilibrium solutions were obtained under static state in half an hour and then the flow time of the solution, namely  $t$ , was measured. The initial concentration of the collagen solution was chosen to be  $1.0 \times 10^{-3}$  g/cm<sup>3</sup>, which was then diluted directly in the viscometer in order to reduce the experiment-to-experiment variability. Considering the volume of the viscometer was limited, it was impossible to measure the flow time of a collagen solution directly diluted down to  $1.0 \times 10^{-5}$  g/cm<sup>3</sup>. A collagen solution with the initial concentration of  $1.0 \times 10^{-3}$  g/cm<sup>3</sup> could be diluted to  $1.0 \times 10^{-4}$  g/cm<sup>3</sup> by adding pure solvent to the viscometer. Subsequently, by removing a volume of collagen solution followed by diluting, its concentration could reach  $1.0 \times 10^{-5}$  g/cm<sup>3</sup> and the flow time of the extremely diluted collagen solution could be measured. After the flow times of the various dilutions of the collagen solutions were measured, the viscometer was immediately washed using pure solvent for 3–5 times. Replacing the viscometer in the bath, the flow time of the pure solvent, namely  $t'_0$ , was measured again. Obviously,  $t'_0$  should be slightly larger than  $t_0$  if polymer was adsorbed on the viscometer capillary during the measurements. After the determination of  $t'_0$  (flow time of the pure solvent after the experiment), the viscometer was soaked in a chromic acid mixture for a day, then rinsed repeatedly with deionized and distilled water, and finally rinsed with boiled deionized and distilled water under ultrasonic vibrations. Then the clean glass viscometer was sent to a common oven to dry at 125 °C. After the flow times were determined three times ( $t_1$ ,  $t_2$ , and  $t_3$ ), the viscometer was again cleaned by above treatments. Then the viscometer was washed with a mixed acid of H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> having a volume ratio of 50:1 and then coated with dimethyl dichlorosilane to render the surface hydrophobic to prevent the solution from being adsorbed on the glass surface of the capillary.<sup>[18]</sup> In the end, the corresponding  $t_0$  (flow time of the pure solvent at beginning of the experiment) and  $t'_0$  (flow time of the pure solvent after the experiment) were determined in the hydrophobically treated viscometer for three times. The flow times of the solvent in the capillary with or without the hydrophobic treatment are shown in Table. 1. The effect of shear rate was characterized at two shear rates for collagen in 0.1 M acetic acid (salt free) solutions of various collagen concentrations and were carried out with the hydrophobic treatment of the capillary surface.

## Results and Discussion

### Effect of Adsorption

The curve of reduced viscosity plotted against concentration showed an upward trend with decreasing concentration; this is the so called as “the abnormal viscosity behavior”. As

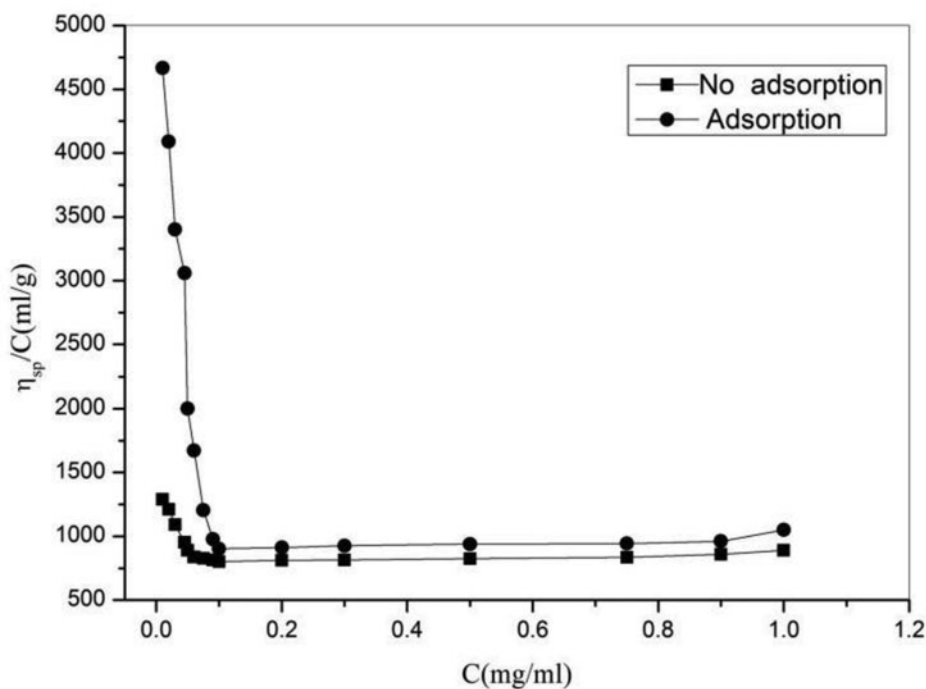
**Table 1**

The flow times of the solvent in the capillary with or without the hydrophobic treatment with dimethyl dichlorosilane

Flow times(s)	Hydrophilic		Hydrophobic	
	$t_0$	$t'_0$	$t_0$	$t'_0$
$t_1$	155.17	158.66	155.19	155.20
$t_2$	155.15	158.67	155.18	155.21
$t_3$	155.14	158.65	155.18	155.19

discussed in a number of reports,<sup>[19,20]</sup> the abnormal viscosity behavior of neutral polymers in extremely dilute solutions results from the adsorption of polymeric solutes on the viscometer capillary walls which reduces the effective radius of the viscometer capillary. Yang et al. have proposed an improved viscosity measurement method to eliminate the abnormal viscosity behavior.<sup>[19]</sup> In general, to obtain the viscosity of a polymer solution, first, the flow time of the pure solvent is measured using a thoroughly cleaned viscometer, namely  $t_0$ . After drying it, second, the flow times of a polymer solution with different concentrations, namely  $t$ , is measured. However, in a careful viscometric study on dilute solutions of water soluble polymers, Cheng et al. had noticed that, even if a used viscometer was cleaned by repeated flushing with water, the flow time of water increased as compared with that of a thoroughly cleaned viscometer.<sup>[21]</sup> Thus, Yang et al. thought that when measuring the flow time of a polymer solution, the viscometer capillary state was not the same as when measuring the flow time of the pure solvent. They suggested that the viscometer should be cleaned by the pure solvent 3–5 times immediately after the flow time of a polymer solution was measured and then to measure the flow time of the pure solvent, namely  $t'_0$ . As a result, the relative viscosity of the polymer solution should be determined by  $t/t'_0$ . This method can be used to reduce the abnormal viscosity behavior of the extremely diluted polymer solution mainly caused by the adsorbed polymer upon the viscometer capillary walls. Table 1 shows that the flow times for pure solvent for each repeated measurement differed when there was no hydrophobic treatment ( $t_0 < t'_0$ ), which evidenced the influence of adsorption. As expected, after the hydrophobic treatment of the surface of the capillary, the flow times were almost equal ( $t_0 = t'_0$ ).

Figure 1 shows the plots of reduced viscosity ( $\eta_{sp}/C$ ) against concentration of collagen acidic aqueous solutions for the different adsorption conditions. It can be seen that when using the untreated viscometer to determine the viscosity of the collagen solution, an upswing of the  $\eta_{sp}/C$  vs.  $C$  curve in acetic acid solution was observed at extremely low concentrations. However, a smaller abnormality of viscosity behavior still existed in determining the viscosity of collagen solution by the treated viscosimeter. It indicated that adsorption was not the only cause of the abnormalities of viscosity behaviors. Collagen is a rod-like, triple helix, and the conformational changes of the collagen helices in the solution were expected to be trivial. Therefore, intermolecular electrostatic forces might be the reason for the residual upswing in viscosity with decreasing concentration. Indeed, the viscosity abnormalities have also been shown to exist in charged microspheres and other rigid particles solution.<sup>[22]</sup>

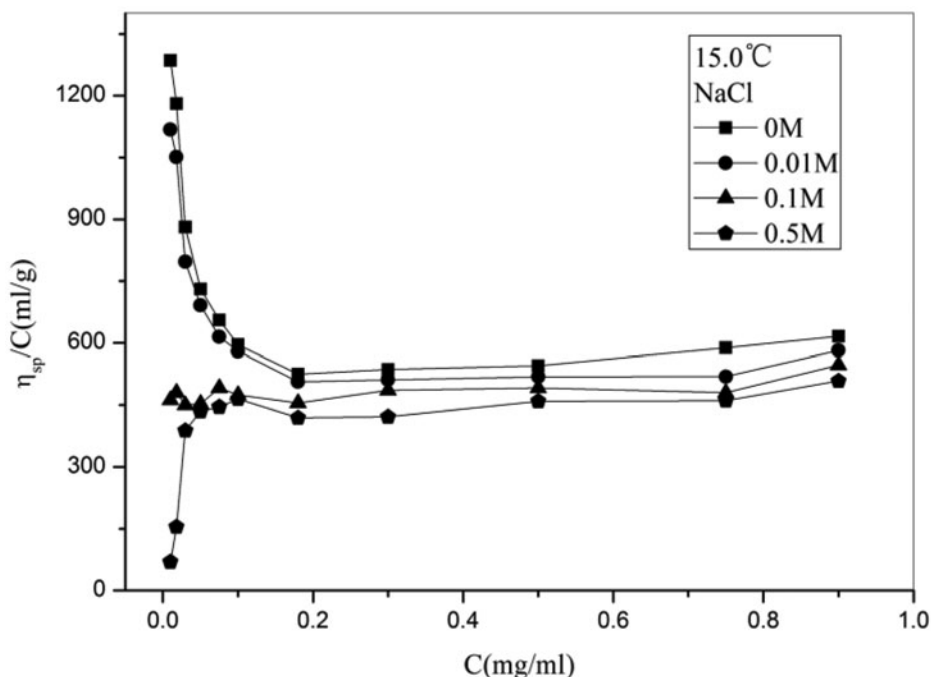


**Figure 1.** Plot of reduced viscosity with respect to concentration of collagen solutions for different adsorption conditions.

### Effect of Salt

Collagen is a typical amphoteric biopolymer since it contains both positive and negative charges due to the functional groups present on the chains of the polymer. Generally, collagen is positively charged in acetic acid solution. In this case, the electrostatic interactions between the positively charged amine functional group has an important effect on the aggregation and structural conformation of collagen molecules. Huelin found that an electric field can stimulate collagen fibrillogenesis in an acidic electrolyte, while unaggregated collagen molecules were preferentially adsorbed to the electrified interface itself.<sup>[23]</sup> The reduced viscosities of collagen acetic acid solution, with the addition of NaCl at different concentrations, were carefully measured with collagen concentrations ranging from  $1 \times 10^{-3}$  to  $1 \times 10^{-5}$  g/cm<sup>3</sup> at 15.0°C in the hydrophobically treated viscometer (Fig. 2); the NaCl concentration was held constant as the collagen was diluted. The results showed that the reduced viscosity ( $\eta_{sp}/C$ ) increased, apparently without limit, with collagen dilution in the nonsalt acetic aqueous solution as well as for the 0.01 M NaCl concentration. However, the reduced viscosity at low collagen concentrations decreased significantly with the addition of 0.5 M salt. The viscosity curve remained nearly flat when the concentration of added NaCl was 0.1 M. The constant viscosity at low concentrations in 0.1 M salt can be interpreted as follows; the arrangement of the rodlike macromolecules in the diluted solutions was readily affected by the intermolecular electrostatic forces. This interaction was shielded by the low molecular weight salt, and the collagen solution showed neutral macromolecules viscosity behavior when the concentration of salt was increased to a certain value (here 0.1 M). The origin of the downturn for 0.5 M salt at low collagen concentration is unknown





**Figure 2.** Plot of reduced viscosity with respect to concentration of collagen solutions for different NaCl concentration.

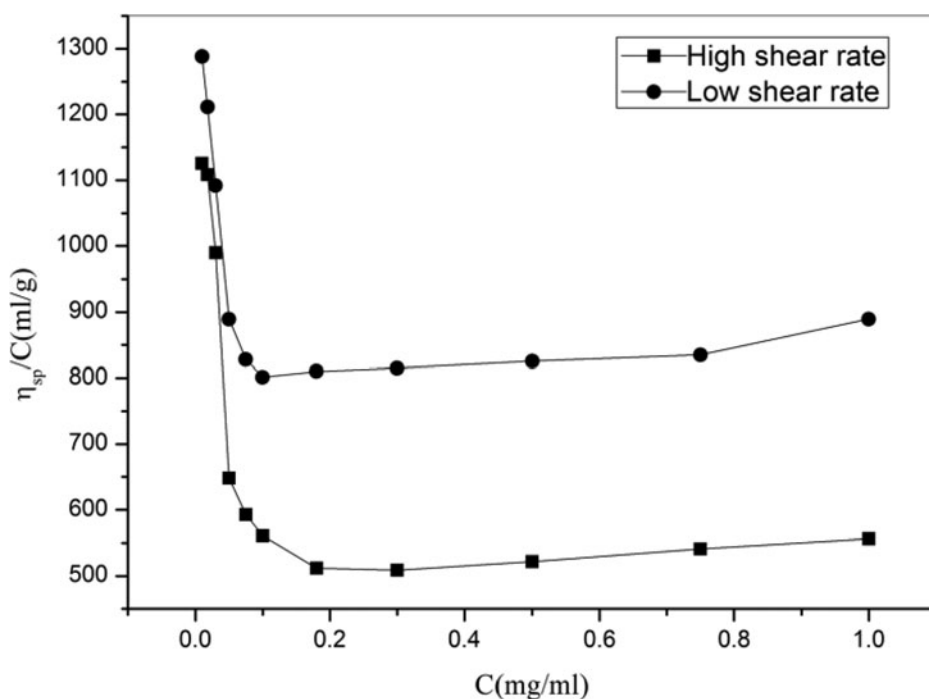
at present and is in need of further study. It may have resulted from the aggregation of collagen molecules.

### *Effect of Shear Rate*

As mentioned earlier, the collagen triple helix is about 1.5 nm in diameter and 300 nm in length;<sup>[8]</sup> light scattering data gives an axial ratio of 228.13.<sup>[15]</sup> Therefore, the collagen solution viscosity is very sensitive to shear rate. Figure 3 shows the plot of reduced viscosity with respect to concentration of collagen in 0.1 M acetic aqueous solution (no salt) at two different shear rates (25°C); the reduced viscosity decreased with the increase of shear rates, indicating a shear-thinning phenomenon. In addition, we also found that the viscosity abnormal behavior with decreasing low-concentration occurred, independent of the changes of the shear rate. Newtonian conditions normally can be achieved in polymer solutions by reducing the rate of shear sufficiently, and under Newtonian conditions, the value of the intrinsic viscosity can be obtained by extrapolation of the reduced viscosity to zero concentration. However, it was impossible to obtain the value of the intrinsic viscosity of collagen due to the presence of the abnormal viscosity behavior in the experiment.

### *Effect of Temperature*

Temperature is an important factor in the study of polymer solution viscosity, Figure 4 shows the plot of reduced viscosity with respect to concentration of the collagen in acetic aqueous solution (no salt) at three different temperatures. The plots indicate the abnormal



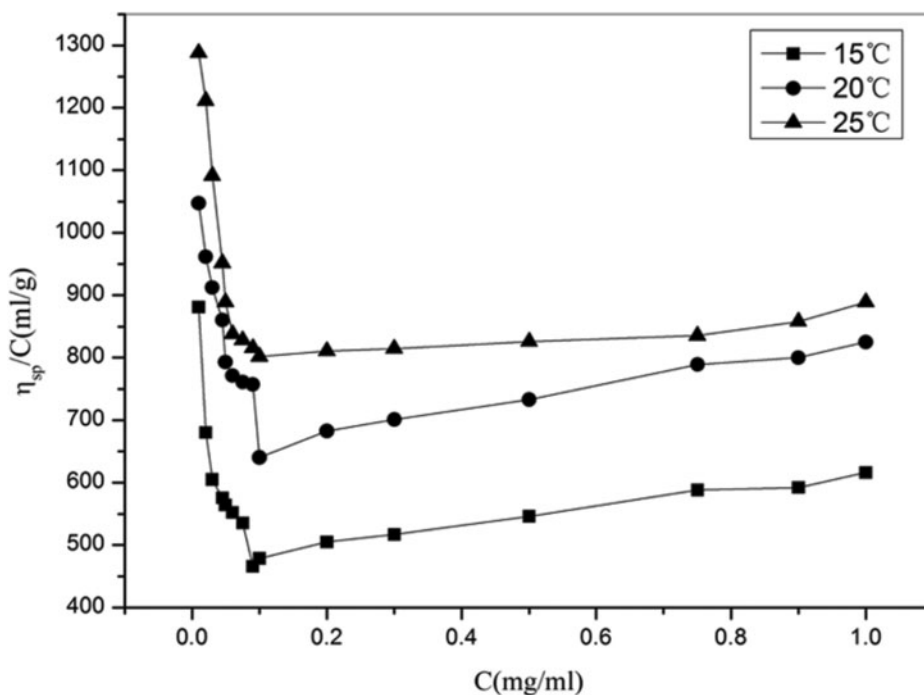
**Figure 3.** Plot of reduced viscosity with respect to concentration of collagen solutions for different rates of shear in the 0.1M acetic acid solvent (salt free, 25°C).

behavior at all three temperatures. Moreover, the intrinsic viscosity increased with an increase of temperature. It has been reported that the intrinsic viscosity of gelatin solutions decreases with increasing of temperature.<sup>[24,25]</sup> Ronzon measured the intrinsic viscosity of acetic acid-soluble collagen with the addition of sodium acetate, and found the intrinsic viscosity values were 2050 ml/mg at 4°C and 2200 ml/mg at 25°C.<sup>[16]</sup> Presumably, he did not see the abnormal behavior due to the added salt. Why does the intrinsic viscosity of collagen solution increase with the increase of the temperature? The suggested answer was that the collagen molecules in dilute solution tend to associate at high temperature due to the enhancement in the hydrophobic interactions among the collagen molecules. Indeed, the collagen molecules have been shown to be prone to aggregate in solution at high temperature.<sup>[26,27]</sup>

## Conclusions

The purpose of this work was to examine the viscosity behavior of collagen in dilute 0.1 M acetic acid aqueous solutions and to study the influence of adsorption on the capillary wall, electrolyte concentration, shear rate, and temperature on the viscosity of the collagen solutions through an analysis of the experimental results. Owing to the limitation of the existing theories of polymer solutions, the explanation can only be given in a qualitative way.

Adsorption was not the only reason for the abnormalities of the viscosity behavior; we suggest it also resulted from the long-range, inter-molecular, electrostatic forces. The reduced viscosity of the collagen solution decreased as the shear rate increased and presented



**Figure 4.** Plot of reduced viscosity with respect to concentration of collagen solutions for different temperatures in the 0.1M acetic acid solvent.

the shear-thinning phenomenon as a result of the high aspect ratio of the collagen molecules. The reduced viscosity of the collagen solutions increased with an increase in temperature, suggested to be due to association of the collagen molecules at high temperature.

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