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Influence of temperature, pH, and ionic strength on the rheological properties of oviductus ranae hydrogels

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The main rheological features of oviductus ranae (OR) hydrogels were investigated as a function of temperature, pH and ionic strength. With respect to the steady shear measurements, a reduction in viscosity was observed as the temperature increased. The flow behavior can be well described by the Herschel-Bulkley model in the down curve at a shear rate of 300 to 0 s^{-1}, where standard error is lower than 20, and it behaved like a viscoplastic fluid. OR gels showed greater extent of thixotropy with the increase of temperature. Temperature dependence on apparent viscosity at a specified shear rate can be described by the Arrhenius model with high R^2 values. The thermostability of OR hydrogels were verified by temperature sweep test, which showed that there was no phase transition observed in the temperature range of 10 to 90°C. It was found that at pH 5 to 10 and the salt concentration of 0 to 10 g/L, the OR hydrogels showed very weakly pH-dependent and salt-dependent rheological properties and dominant elastic behavior. All data indicated that OR has great potential for application as gelling and thickening agent with multiple healthy functions.

Key words: Rheological properties, oviductus ranae hydrogels, temperature, pH, ionic strength.

INTRODUCTION

Oviductus ranae (OR), a traditional Chinese medicine and functional food, is made from dried oviduct of the female forest frog, *Rana chensinensis*. As OR contains many nutrients and functional components such as proteins, polysaccharide, fat, and physiological activators (e.g. estradiol, progestin, and testosterone) (Liu and Liu, 2007; Zheng et al., 2008; Hu et al., 2003), it has been reported and recorded in '*Compendium of Materia Medica* and *Chinese Pharmacopoeias'* for the functions of anti-fatigue (Chen et al., 2005), antioxidation (Li et al., 2008), regulating blood fat (Yang et al., 2011) and invigorating the kidney (Hu et al., 2003).

When OR contacts with water, the biopolymers unfold,

exposing the reactive surfaces of neighboring biopolymers molecules, which then interact to form intermolecular bonds. When sufficient bonding (hydrogen bonds, ionic linkages, hydrophobic interactions, and covalent bonds) occurs, it expands and transforms into hydrogel with three-dimensional cross-linked hydrophilic biopolymer network that can absorb large quantity of water. The reason why OR can be turned into hydrogel is attributed to its most important feature of multicomponent nature. This system is made up of two major kinds of biopolymers: protein and polysaccharide. Many proteins/polysaccharides are hydrocolloids and considerable interest has been devoted to the study of polysaccharide-protein

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Table 1. Amino acid composition of oviductus ranae (as g amino acid/ 100 g OR).

Amino acids	% of amino acid	Amino acids	% of aminoacid
Asp	4.00	Cys	ND
Thr	6.66	Leu	2.10
Ser	2.76	Tyr	1.80
Glu	3.81	Phe	1.40
Gly	1.81	His	4.86
Ala	0.75	Lys	3.35
Val	1.74	Arg	1.33
Met	0.39	Pro	3.48
Ile	2.04	H-Pro	ND

ND for not detected.

mixtures. The structural functions of these biopolymers are greatly affected by their interactions with each other and with other components in food systems (Girard et al., 2002; Andrade et al., 2010; Kerry et al., 1999; van den Berg et al., 2008; Turgeon et al., 2003; Agbenorhevi and Kontogiorgos, 2010; Ribotta et al., 2007; Mohamed and Xu, 2003; Gaaloul et al., 2009). Because of their ability to modify the rheological and functional properties of food systems, they can be used in the food industry for changing texture such as gel formation, water retention, emulsification and aroma retention. Moreover, OR is also attracting the increasing attention of pharmaceutical and functional food industry, not only because of its multiple beneficial effects on human health mentioned above, but also its thickening or gelling properties.

As compositionally and structurally complex materials, hydrogels exhibit a wide range of rheological properties under different conditions. And those properties are strongly affected by temperature, pH, and ionic strength (Tang et al., 2007; Lai et al., 2008; Romero et al., 2009; Venugopal et al., 2002; Cakir and Foegeding, 2011). To our surprise, however, there is no report concerning the rheological characteristics of OR hydrogels influenced by factors mentioned above to date. Therefore, in this work, the influences of temperature, pH, and electrolyte (NaCl and $CaCl_2$) conditions on rheological properties of OR were extensively investigated using both steady shear and small amplitude oscillatory measurements based on our previous corresponding study (Liang et al., 2012). Our work may give an insight into the dependence of molecular structure on specific conditions (such as temperature, pH, ionic strength of the system) and reveal the rheological properties of OR hydrogels as a new source of hydrocolloid gum.

MATERIALS AND METHODS

Proximate composition and amino acid analysis of oviductus ranae

OR was purchased from Antu Baoli health food Co., Ltd., China, and it was produced in Changbai Mountain of Jilin province in Northeast China. The moisture (11.9±0.2%), ash (4.5±0.1%), fat (2.0±0.1%) and crude polysaccharide (13.1±0.1%) contents of OR was determined according to the method as described by AOAC (AOAC, 1990; Dubois et al., 1956). The crude protein (62.0±0.1%) content was determined by estimating its total nitrogen content by Kjeldahl method (AOAC, 1990). A factor of 5.8 was used to convert the nitrogen value to protein. Amino acid composition of OR was determined after hydrolyzation in 6N HCl/0.1% mercaptoethanol solution for 24 h at 110°C. The sample in aminoethyl cysteinyl dilution buffer was then analyzed using amino acid analyzer (S-433D, Sykam, Amtsgericht Augsburg, Germany) (Table 1).

Preparation of oviductus ranae hydrogels

The sample of dry OR was ground in porcelain mortar and then sifted through a 40 mesh sieve. Deionized water was used to mix with the powdered OR to form hydrogels. The hydrogels were prepared at 1:80 (OR: water, w/w, sample A), 1:120 (B), 1:160 (C), and 1:200 (D) concentrations. The OR powder was mixed with pHs 4, 5, 6, 7, 8, 9, 10, 11 water solutions (1:120) to get corresponding hydrogels with different pH value respectively. The pH values were adjusted by 0.1 M NaOH or HCl. The OR powder was suspended in 0, 0.05, 0.1, 1 and 10 g/L NaCl or $CaCl_2$ solutions to get corresponding hydrogels with different electrolyte concentration. Different ionic strengths were obtained by adjusting with 200 g/L NaCl or $CaCl_2$. All the above samples were allowed to equilibrate for 24 h at room temperature (20°C). In order to get rid of entrapped air bubbles and acquire homogeneous samples, gentle stirring was imposed on the hydrogel samples. Then, they were allowed to equilibrate for another 24 h at 20°C to complete hydrogels structure formation.

Rheological tests

Rheological measurements were performed on a controlled-stress rheometer (AR500, TA Instruments, Texas, USA) and equipped with an aluminum parallel plate geometry (40 mm diameter, 1 mm gap). Each kind of rheological experiment was performed at its corresponding temperature, and the temperature was controlled by a water bath connected to the Peltier system in the bottom plate. A thin layer of silicone oil was applied on the surface of the samples in order to prevent evaporation. The linear viscoelastic (LVE) region was determined for each sample through a frequency strain-sweep measurement at 1 Hz and 20°C (amplitude range is 0.01 to 100%, data not shown). Viscoelastic properties of OR hydrogel samples were determined within the linear viscoelatic region. An equilibration of 3 min was performed before each measurement.

Shear stress-shear rate data were obtained at 20°C on the samples (A, B, C or D). The instrument was programmed for set temperature and equilibration followed by two-cycle shear in which the shear rate was increased linearly from 0 to 300 s⁻¹ in 3 min (up curve) and immediately decreased from 300 to 0 s⁻¹ in the next 3 min (down curve) at 5, 25, 50, 75 or 95°C. This process was repeated two more times for each sample. Data from the descending segments of the shear cycle were used to characterize the flow of the samples and to estimate the Herschel-Bulkley (H-B) parameters by using the equation:

$$\tau = \tau_0 + K\dot{\gamma}^n \tag{1}$$

Where, τ_0 is yield stress, K is the consistency index, and n is the flow behavior index. The dimensions of K depWhere, τ_0 is yield stress, K is the consistency index, and n is the flow behavior index. The dimensions of K depend upon the value of n. The physical meaning of K and n (<1) in equation is similar to the equation of power-law. With the use of the parameter τ_0, this model provides a somewhat better fit to some experiental (Chhabra and Richardson, 2011).

The influence of temperature on the viscosity at 100 s⁻¹ for non-Newtonian fluids of each concentration could be expressed by the Arrhenius model:

$$\eta = A\exp^{(Ea/RT)} \tag{2}$$

Where, A is the frequency factor or viscosity coefficient at a reference temperature (Pa.s), E_a is the activation energy (kJ/mol), R is the gas constant (kJ/mol K) and T is the absolute temperature (K). Activation energy can be determined from the slope of lnη versus 1/T plot.

The thermostability of the samples were determined according to the procedure of Tischer et al. (Tischer et al., 2006) with small changes. After the sample was equilibrated at 10°C for 2 min, the temperature of the sample was increased to 90°C and subsequently its temperature was decreased to 10°C with a ramp rate of 5°C/ min for both heating and cooling. A sweep of this temperature profile is performed one more time. In the tests a constant frequency of 1 Hz and strain amplitude of 1% were used.

The viscosity and flow properties were measured for hydrogels (1:120) with different pH values (3.0 to 11.0) and salt concentrations (0 to 10 g/L) at shear rate of 50 s⁻¹ and constant temperature of 20°C. Small amplitude oscillatory tests were also performed at 20°C over the frequency range of 0.1 to 10 rad/s. The strain amplitude for the frequency sweep measurements was selected as 1%, which was in the linear viscoelastic region for all samples. The mechanical spectra were obtained through recording storage modulus (G'), loss modulus (G"), and loss tangent (tan δ = G"/G') as function of angular frequency.

Statistical analysis

The experimental results obtained from this study were fitted to different kinetic and mathematical models using TA Rheology Advantage Data Analysis software V 5.4.7 (TA Instruments, UK). The fit and estimates were calculated at a significance level of 95%. The best fit regression model could be selected on the basis of standard error (SE), which is defined as:

$$\frac{\sum (X_m - X_c)^2 / (n-2)^{0.5}}{Range} \times 1000 \tag{3}$$

Where, X_m is the measured value; X_c is the calculated value; n is the number of data points and $range$ is the difference between the maximum value of X_m and the minimum value.

RESULTS AND DISCUSSION

Effect of temperature

The effect of temperature on the flow behavior is shown in Figure 1. There was a reduction in stress (viscosity) as the temperature increased. The experimental data of shear stress versus shear rate for the OR hydrogels at different concentrations and temperatures were fitted by H-B model. The consistency index (K), the flow behavior index (n) and the yield stress. (τ_0) as influences of OR concentrations and temperatures were derived from the flow curves of OR hydrogels (Table 2) by fitting the descending flow curve with the H-B model ($SE < 20$). H-B model is often used to describe the flow behavior of food gels which belong to viscoplastic materials showing shear-thinning behavior at stress levels exceeding τ_0 (Wang et al., 2011). The yield stress (τ_0) represents a finite stress required to initiate flow. The τ_0 values of the samples determined using H-B model approximately show the trend with concentration and temperature: yield stress increased with increase of concentration and decreased with increase of temperature. The K values have similar trends which relates to the capacity of water binding. The thixotropy of OR hydrogels at various temperature are also presented in Figure 1. For the range of shear rates used in this study, the H-B model describes flow behavior of each sample. Appearance of a hysteresis area in the plot of shear stress versus shear rate means that all hydrogels at different temperature exhibit time-dependent behavior. And the larger the enclosed area, more severe is the time-dependent behavior of the materials. All the samples exhibit clockwise hysteresis loop, indicating that shear stress obtained from the increasing-order of shear rate (up curve) is much larger than the corresponding value of the decreasing-order of shear rate (down curve), which suggests thixotropic behavior of the hydrogels. At low shear rate of up curve, the shear stress increase quickly with increase of shear rate. So, the hydrogels exhibited extremely thixotropic behavior, which manifests as a regional peak in the ascending curve of hysteresis loop, neither the H-B nor any other typical viscous model could fit well to the ascending flow curve. Therefore, only the parameters obtained by fitting the descending flow curve were reported in the Table 2.

The clockwise loops can be interpreted as structure breakdown by the shear field (thixotropic behavior) to alter a structure or form a new structure. Some authors have determined thixotropic behavior by the coefficient of thixotropic breakdown, K_d, which is defined as the ratio of the hysteresis area to the area beneath the ascending shear curve (Dokic et al., 2010):
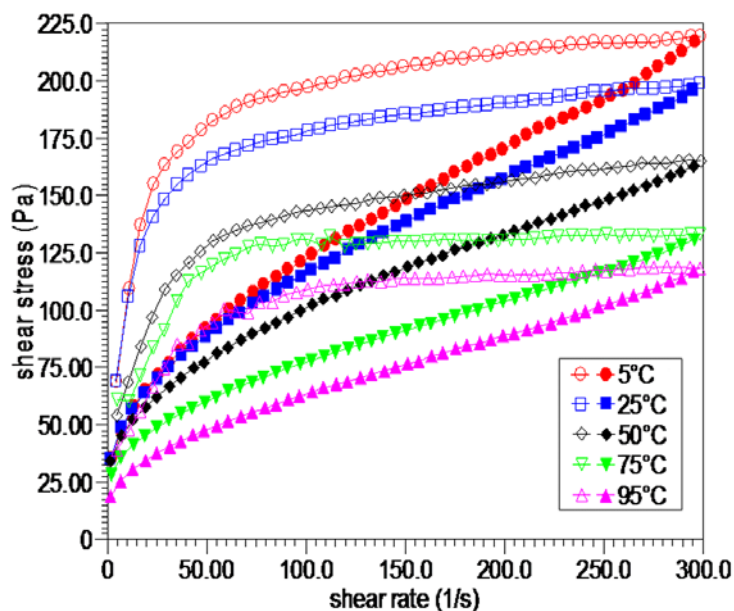
$$K_d = \frac{A_{up} - A_{down}}{A_{up}} \tag{4}$$

Figure 1. Flow curves of 1:120 (w/w) oviductus ranae hydrogel at various temperature. Open symbols, up curve; closed symbols, down curve.

Table 2. The Herschel-Bulkley equation parameters for oviductus ranae hydrogels at different concentrations and temperatures (downward curve).

Samples	Temperature (°C)	K_d	Down curve			
			τ_0^A (Pa)	K^B (Pa·s^n)	n^C	SE
A	5	0.3052	49.78	7.610	0.6264	10.56
	25	0.2754	44.66	6.933	0.5883	8.087
	50	0.2546	42.54	6.447	0.5971	8.604
	75	0.3241	39.09	3.844	0.6529	8.091
	95	0.3681	21.37	3.332	0.6747	13.02
B	5	0.2903	31.96	5.873	0.6272	7.991
	25	0.2587	31.58	5.585	0.5908	6.375
	50	0.2194	30.73	5.050	0.5523	5.625
	75	0.2953	28.48	3.330	0.6187	8.504
	95	0.2977	18.12	3.051	0.5932	9.289
C	5	0.2796	22.86	5.595	0.5823	7.701
	25	0.2573	21.21	4.278	0.5877	7.127
	50	0.2158	19.41	3.609	0.5506	4.443
	75	0.2656	16.59	2.699	0.6300	10.62
	95	0.2782	15.29	2.023	0.6081	8.953
D	5	0.2400	16.17	4.139	0.5926	6.905
	25	0.2254	15.10	3.745	0.5743	7.269
	50	0.2019	14.14	2.707	0.5906	7.101
	75	0.2328	12.19	2.301	0.5844	5.855
	95	0.2645	10.83	1.091	0.6685	13.84

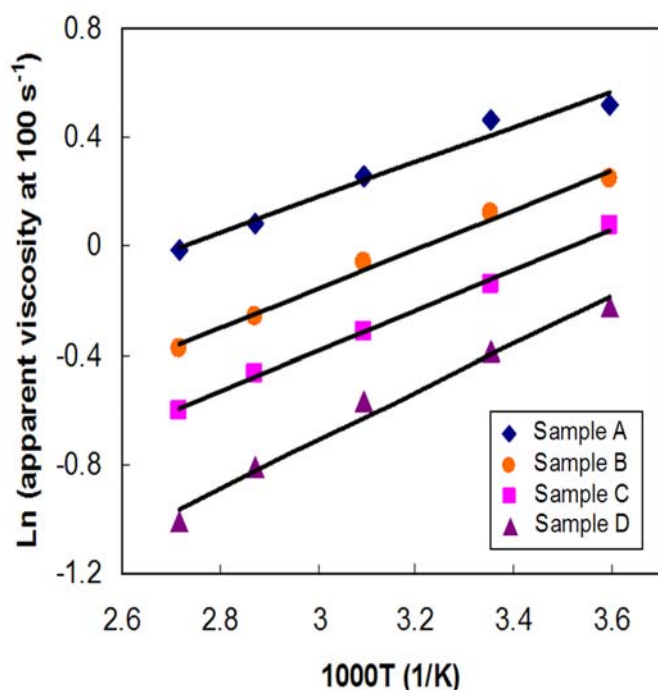[A] Yield stress, [B] Consistency coefficient, [C] Flow behavior index.

Figure 2. Arrhenius plot for the apparent viscosities at the shear rate of 100s^{-1}.

Table 3. Arrhenius model (η=Aexp$^{(Ea/RT)}$) for temperature dependency of apparent viscosity (at 100 s^{-1}) for OR hydrogels at different concentrations.

Samples	A (Pa s)	E_a (kJ/mol)	R^2
A	0.173	5.362	0.972
B	0.163	6.014	0.992
C	0.073	6.207	0.995
D	0.035	7.316	0.979

Where, A_{up} and A_{down} are the areas under ascending and descending flow curves, respectively.

Table 2 shows the data of K_d at the different temperatures and concentrations studied. It was found that K_d increased as the concentration of the extract increased, which means an increased extent of structuring of the samples with concentration. Furthermore, K_d is greater at higher temperatures for all the concentrations studied. Since a coefficient of thixotropic breakdown is an index of the energy needed to destroy the structure of the system, it can also be explained as a result of increased intermolecular interaction as suggested previously for the greater concentration dependence of viscosity at higher temperatures (Karazhiyan et al., 2009).

An increase in temperature from 5 to 95°C did not

significantly influence the critical shear rate limiting the H-B region or the shape of the OR hydrogels, hence the H-B flow index (*n*) hardly changed sharply (Table 2). It must be emphasized that the shear thinning properties were still clear enough at 95°C as pointed out by the H-B flow index value of 0.59 to 0.67. The flow behavior index indicated that at higher temperature the systems were obvious pseudoplastic which was confirmed by K_d.

The effect of temperature on apparent viscosity at a specified shear rate can be described by the Arrhenius relationship (Eq. (2)), Figure 2 illustrates that the change of apparent viscosity with temperature generally obeyed the Arrhenius model. Frequency factors (*A*), activation energies (*E$_a$*) and coefficients of determination (R^2) are show in Table 3. Good agreement with linearity was found. The activation energy indicates the energy barrier that must be overcome before the elementary flow process can occur (Rao, 2007). The magnitude of the temperature effect varied with concentration as shown by the E_a values in Table 3. E_a decreased and constant *A* increased with the increase in OR concentrations. This effect is reversible and it is due to the interactions of the molecules in system which varies with the temperature. As showed in Table 3, the activation energy for the OR hydrogel decreased from 7.316 kJ/mol at 1:200 concentration to 5.362 kJ/mol at 1:80. This indicates that the higher the activation energy, the greater the effect of temperature on the viscosity. Therefore, as temperature increases, the thermal energy of the molecules varies and the intermolecular distances change.

To assess the thermostability of the OR hydrogels, a temperature swing test is an effective method in terms of physical stress because by changing the temperature in a defined manner conditions can be simulated. If the moduli *G'* and *G"* have identical values at recurring temperatures at constant frequency and amplitude (in the linear viscoelastic range) regardless of the number of temperature sweeps performed, a sample can be classified as stable. The temperature dependences of *G'* and *G"* of the sample A are shown in Figure 3 (the curves of other samples are similar to the sample B, so they are not shown). The curves for the storage and loss module were nearly parallel through the given temperature range and the storage modulus *G'* was always larger than the loss modulus *G"* over the entire measuring window. If the storage modulus increases, so do the loss modulus. These experimental phenomena are beyond our expectation, because the macromolecules of proteins always denature in the environment of high temperature. If the denaturation happens, the moduli *G'* and *G"* would change sharply. But the fact is not so which indicates that OR hydrogels are thermostable. Among many gels, there is always a phase transition from a liquid to gel or adversely with the change of temperature (Moraes et al., 2009; Chiou et al., 2006). But the sol-gel transition cannot be seen in the temperature range of 10 to 90°C which
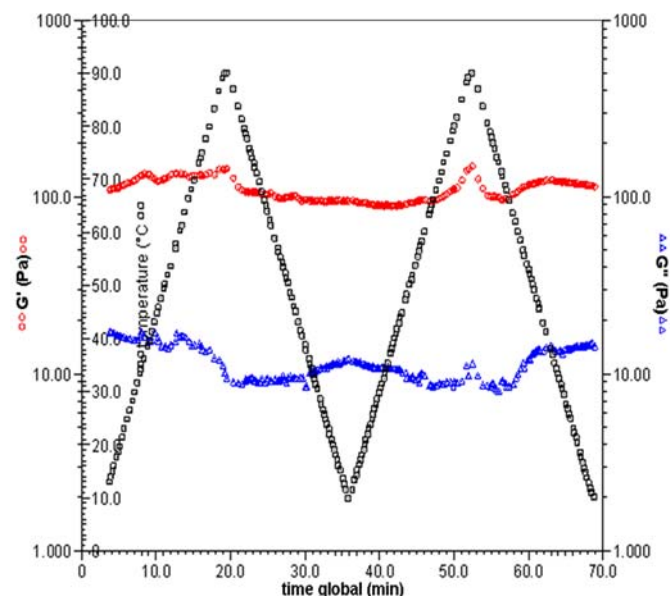
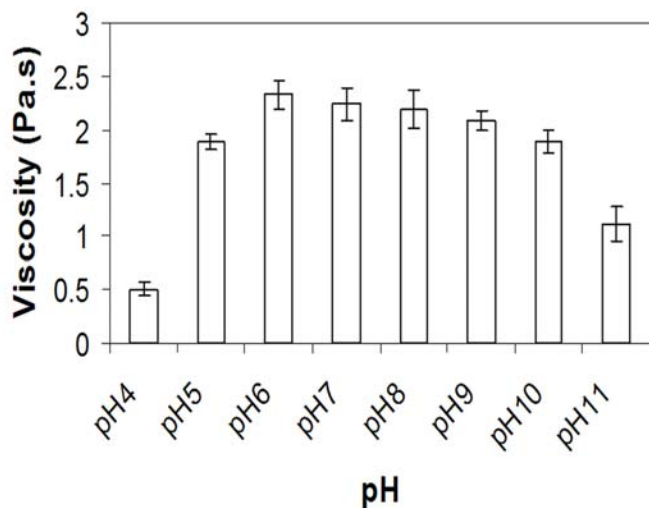**Figure 3.** Dynamic swing test for 1:120 (w/w) oviductus ranae hydrogel.

Figure 4. Influence of pH on steady flow viscosity of 1:120 (w/w) oviductus ranae hydrogels at the shear rate of 50 s^{-1}.
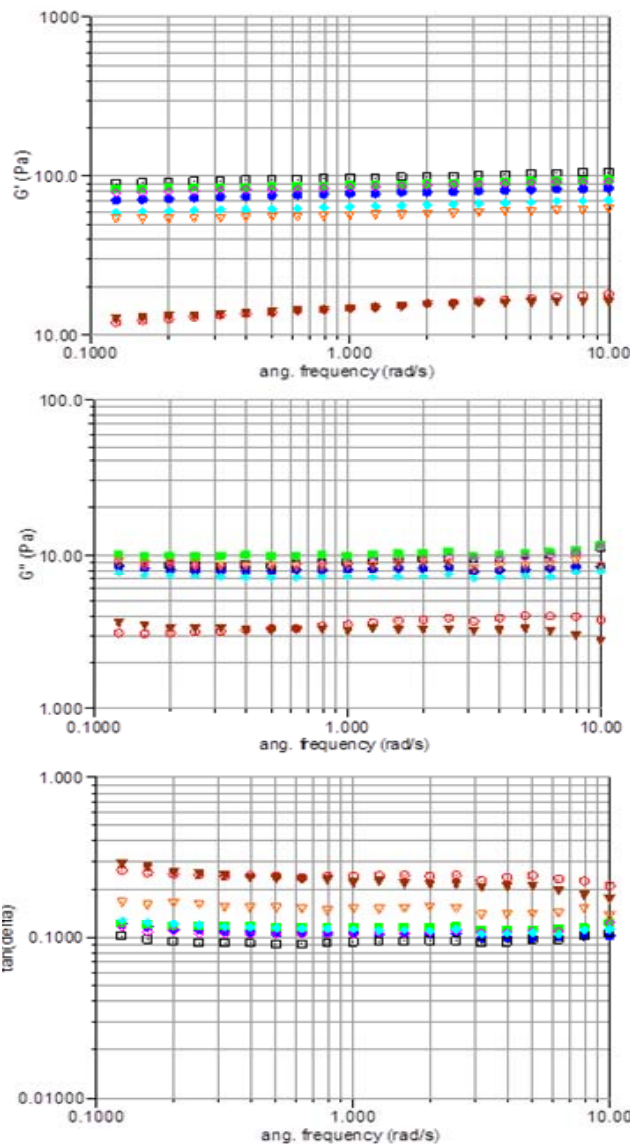

Figure 5. Dependence of storage modulus (G'), loss modulus (G''), and loss tangent (tan δ = G''/G') on   frequency (f) for Oviductus Ranae hydrogels at different pH. (○) pH4; (●) pH5; (□) pH6; (■) pH7; (◇) pH8; (◆) pH9; (▼) pH10; (▽) pH11.

indicates that, for the samples of OR hydrogels, there is no obvious phase transition.

Influence of pH on the rheological properties

Changes in pH may influence the viscosity of OR. The effect of pH (4 to 11) on the steady flow viscosity (at the shear rate of 50 s^{-1}) of OR hydrogels (1:120) is displayed

in Figure 4. The viscosity was found to be fairly stable over a wide range of pH (5 to 10) and decreased both at lower (pH < 4) and higher (pH > 11) pH which presented the initial and ultimate pronounced decrease of the viscosity at more acidic or alkaline conditions. The influence of pH on the viscosity of different protein-polysaccharide systems has been reported in some literatures (Totosaus et al., 2002; Bazinet et al., 2004). But the similar phenomenon with that in this study is scarce. The G', G'' and tan δ values for hydrogels were also dependent on pH (Figure 5). And there have been some reports on the viscoelastic properties of protein-
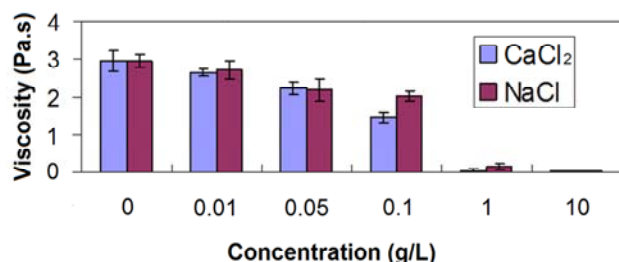
Figure 6. Influence of NaCl and CaCl$_2$ concentrations on steady flow viscosity of 1:120 (w/w) Oviductus Ranae hydrogels at the shear rate of 50 s^{-1}.

polysaccharide systems (Miquelim et al., 2010; Tadpitchayangkoon et al., 2010; Romero et al., 2009; Ribotta et al., 2007; Bazinet et al., 2004; Mohamed and Xu, 2003; Gaaloul et al., 2009). From Figure 5, it can be seen that *G'* and *G''* display the similar changing tendency as with pH change. The *G'*–frequency curve had decreased values at both acidic (pH 4) and alkaline (pH 10) values which is consistent with the influence of viscosity. Moreover, *G'* is more sensitive to the effect of pH than *G''* and *G'* is always greater than *G''* at pH 4 to 11, indicating that OR hydrogel shows dominant elastic behavior. At pH values below 4 (including pH 4) the storage modulus decreased sharply. This is probably due to increased net positive charge in the chains, which could inhibit junction zone formation and therefore result in declined gel rigidity. Above pH 11, the storage modulus also decreases obviously, probably due to deamination of acid amides and changes in the charge density to give a high net negatively charged biopolymer and high repulsive force of protein molecules. The increased charge density can oppose the ability of chains to make contact and form junction zones and thus decrease the gel stiffness. So, the pH has a paramount importance in controlling the electrostatically mediated interaction between proteins and polysaccharides as it can directly control the degree of ionization of the charged groups carried by these two biopolymers (Schmitt et al., 1998).

The pH–tan δ relationship also showed high points at both acidic and alkaline pH with maximum values. As tan δ values represent the *G''/G'* values, a low tan δ value indicates a stronger role for *G'* in the network formed and can represent the relative elasticity of the network. As a result, the lower tan δ values from pH 5 to 9 would suggest better networks. While the low *G'*, high tan δ values would suggest very little biopolymers interaction. The reduction in *G'* also suggests that the OR could be degraded or chemically changed at extreme pH values.

Influence of ionic strength

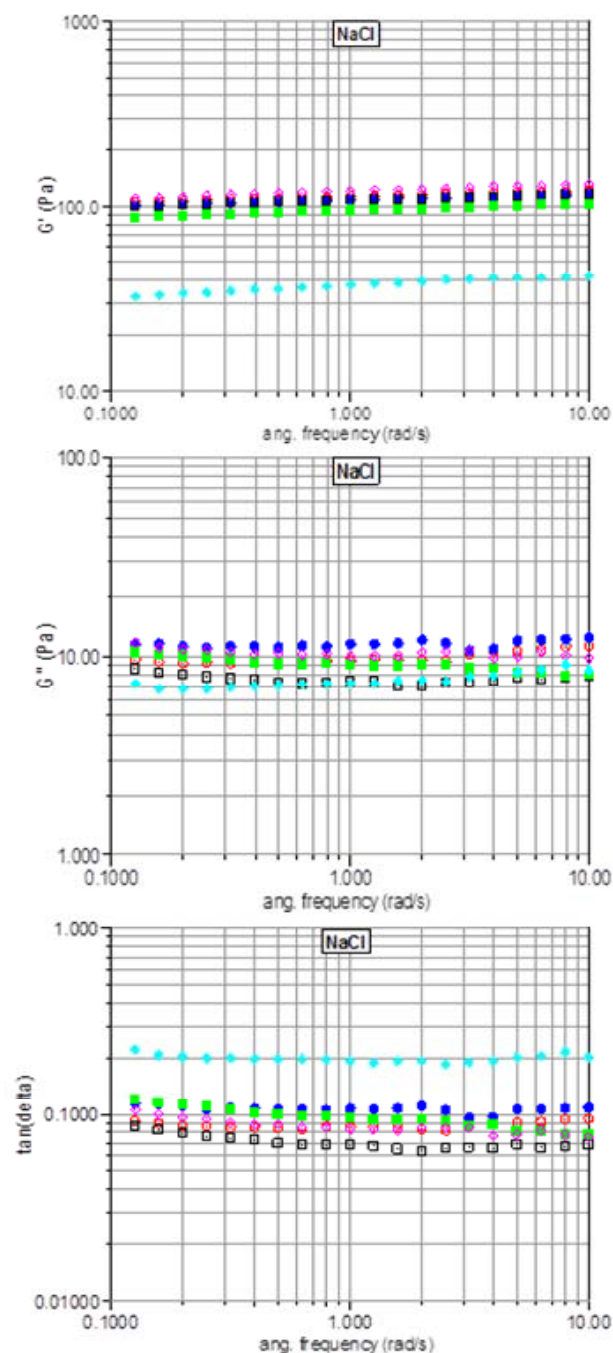Considering salts' effect on the rheological properties, we

Figure 7. Dependence of storage modulus (*G'*), loss modulus (*G''*), and loss tangent (tan δ = *G''/G'*) on frequency (*f*) for Oviductus Ranae hydrogels at different NaCl concentrations. (○) 0 g/L; (●) 0.01 g/L; (□) 0.05 g/L; (■) 0.1 g/L; (◇) 1 g/L; (◆) 10 g/L.

studied monovalent (that is, NaCl) and divalent (that is, CaCl$_2$) salt concentration on the steady flow viscosity (at the shear rate of 50 s^{-1}, Figure 6) and viscoelasticity of OR hydrogel (Figures 7 and 8). As expected, the increase
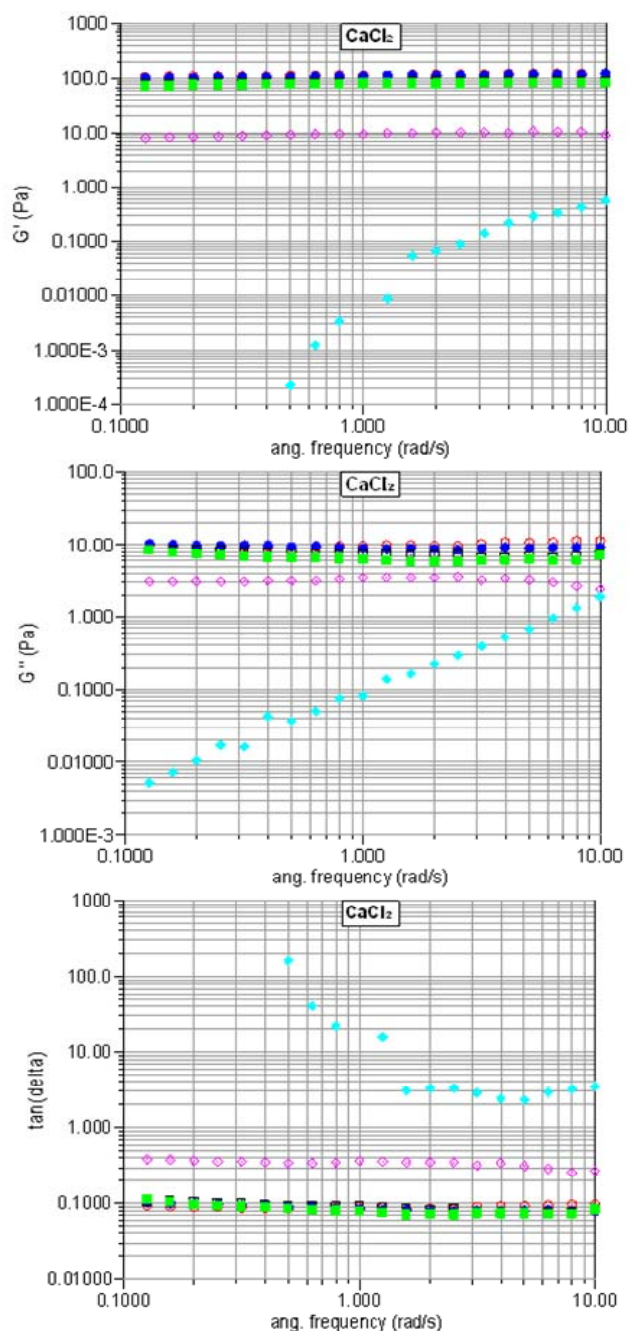
Figure 8. Dependence of storage modulus (*G′*), loss modulus (*G″*), and loss tangent (tan δ = *G″*/*G′*) on frequency (*f*) for Oviductus Ranae hydrogels at different CaCl₂ concentrations. (○) 0 g/L; (●) 0.01 g/L; (□) 0.05 g/L; (■) 0.1 g/L; (◇) 1 g/L; (◆) 10 g/L.

of salt decreased the apparent viscosity and dynamic modulus at high concentrations, and significant ionic selectivity was observed. At salt concentration (NaCl and CaCl₂) higher than 1 g/L, it can be seen that the presence of electrolyte causes a drastic decrease of the viscosity.

And more obvious influence on viscosity can be seen from the change of CaCl₂ concentration. A decrease of viscosity in salt solutions is a common effect in protein-polysaccharide systems. The decrease arises from the screening effect of the charges of the macromolecules due to small ion-pairing. This effect reduces the number of protein molecules which is able to interact with the polysaccharide chains (Matsunami et al., 2007) and can cause the decrease of the three-dimensional network strength, resulting in the decrease of the viscosity of OR hydrogel.

The influence of ionic strength on OR hydrogel properties was also evaluated by dynamic oscillatory rheometry. At the NaCl concentration (C_{NaCl}) lower than 1 g/L or CaCl₂ concentration (C_{CaCl2}) lower than 0.1 g/L, an invarability in *G′*, was observed. When higher concentrations (C_{NaCl} > 10 g/L and C_{CaCl2} > 1 g/L) of salt were added, the storage modulus rapidly decreased. It was initially thought that the network structure would become weaker since salt addition could have led to decreased attractive interactions among biopolymers due to the salting-in effect on proteins. The effect could promote exposure of new regions of the protein surface to the solvent, enabling new electrostatic interactions to occur. It is well known that low concentrations of salt only have a small effect on coacervation of electrostatically stabilised complexes. Higher ionic strengths, on the other hand, prevent complexation due to a reduced entropic driving force from the release of counter-ions (Schmitt et al., 1998).

With the addition of electrolytes, the OR hydrogel is dominant elastic fluid (*G′*,> *G″*, tan δ < 1) at C_{NaCl} used; but at C_{CaCl2} > 10 g/L, it becomes to dominant viscous fluid (*G′* < *G″*, tan δ > 1), indicating that the OR could not form a gel. When divalent ions were used, suppression of coacervation occurred at lower ionic strength values than for monovalent ions. Thus, ionic strength dependence for complex formation between protein and polysaccharide might not only be related to salt concentration, but to the type of ion.

Conclusion

In this study, the influences of temperature, pH and electrolytes (NaCl and CaCl₂) on the viscosity and dynamic modulus (*G′* and *G″*) of OR were examined. With the increased of temperature from 5 to 95°C, it was found that the shear stress (apparent viscosity) of the samples decreased. H-B model was suitable for representing the flow behavior of the tested samples and it was suitable for predicting the yield stress of the samples. Though, the low apparent viscosity was observed with increasing of temperature, the samples still present great extent of thixotropy, which indicates that the inter-macromolecular interactions are still significant at high temperature.

Magnitudes of consistency index (K) and apparent viscosity ($\eta_{a, 100}$) for the OR hydrogels were influenced by temperature and their concentrations. The effect of temperature on $\eta_{a, 100}$ is described well by Arrhenius relationship with high correlations (R^2). As concentration increased, the activation energies and frequency factors decreased and increased, respectively. Temperature sweep test showed that both G' and G'' were nearly independent on temperature, which suggested that the OR hydrogels' are superior to thermostability. At pH 5 to 10 and electrolyte concentration 0 to 10 g/L, the rheological properties of OR show very weak pH and salt dependence, respectively, and dynamic measurements also suggested that elastic behavior dominates throughout the entire frequency range examined. Thus, the gel-like behavior of OR hydrogel was relatively dependent on conditions of solvent (that is, pH, ionic strength). All data indicated that OR, a hydrogel with multiple healthy functions, has unique rheological properties and could be developed as a new source of gelling and thickening agent in modern food industry.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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