

pH Dependence of Rheological Properties of the Mixture of Gelatin and Agar

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Rheological properties of the mixture of gelatin and agar, such as viscosity and elasticity, are affected by the mixing ratio of gelatin and agar, pH, type of gelatin, ionic strength, and so on. In this study, we measured the dynamic viscoelasticity and the turbidity of the mixture over a wide pH range. Furthermore, the same measurements were carried out also at a high ionic strength. Gelatin and agar were completely dissolved in Good's buffer by using an autoclave at the concentrations of 8% and 1.5%, respectively. The mixture formed a transparent and weak gel at pH below the pI of gelatin, while it formed an opaque and elastic gel at pHs above the pI of gelatin. Large effects of ionic strength on the viscoelasticity and the turbidity for the mixture were observed at pH not greater than gelatin's pI.

Key words: gelatin, agar, viscoelasticity, pH, ionic strength

1. INTRODUCTION

Gelatin is a protein produced by partial hydrolysis of collagen and is poly-cationic below its isoelectric point (pI). Agar is a heterogeneous mixture of agarose and poly-anionic agaropectin with acidic side-groups such as sulfate and pyruvate. Therefore, these oppositely charged polymers can form complex via electrostatic interaction under suitable pH conditions. Gelatin and agar are known to form thermo-reversible physical gels which have very different rheological properties. The typical gelling and melting temperature for gelatin are 15 ~ 20°C and 20 ~ 30°C, respectively. On the other hand, agar has a large hysteresis between its gelling temperature (35 ~ 40°C) and melting temperature (75 ~ 85°C). It has been known that the rheological properties of the mixed gel of gelatin and agar are more interesting than those of the individual components [1-3]. Mixed gels have been widely used as synthetic food materials. Texture of food materials is one of the factors to determine its palatability, as well as color, taste and flavor. Texture of mixed gels can be greatly improved by compensating each other's demerits. In bacteriological and bioengineering fields, the mixed gels has been utilized as a soft culture medium to distinguish from motility mutants by their colony morphologies [4], and recently as a hybrid scaffold to provide stable cell adhesive matrices [5, 6].

The rheological properties of the mixture of gelatin and agar, such as viscosity and elasticity, are affected by the mixing ratio of gelatin and agar [3,7], pH [8], the type of gelatin [3], ionic strength [9], and

so on. In this report, we studied the storage modulus (G') and the loss modulus (G'') of the mixture at pH 3.5, 5.6, 7.1, 8.7 and 10.1. Furthermore, the same measurements were carried out at a high ionic strength.

2. EXPERIMENTAL

Gelatin purchased from Nitta Gelatin was derived from lime-cured tissue and the type B with pI 4.8~5.2. Gelatin and agar (Bacto™ Agar, BD) individual solutions were prepared to completely dissolved 8 wt% gelatin and 1.5 wt% agar in 0.1M Good's buffer (MES for pH 3.5 and 5.6, HEPES for pH 7.1, Bicine for pH 8.7 and CHES for pH 10.1) by using an autoclave, respectively. Mixed solutions of gelatin and agar were prepared to simultaneously dissolve both of 8 wt% gelatin and 1.5 wt% agar in Good's buffer as the same manner. In the experiments to examine effects of ionic strength, the same solutions prepared with Good's buffer containing 0.5M NaCl were used.

Measurements of dynamic viscoelasticity were performed by using a dynamic rheometer (Rheograph-Sol, Toyoseiki). G' and G'' were obtained at 30min after lowering the temperature from 65°C to 15°C. The sample was subjected to 3Hz of sinusoidal shear oscillations with amplitude of 50 μ m. Turbidity (τ) measurements were made by using a spectrophotometer (UV-1600, Simadzu). τ at 700nm were obtained in sol state at 65°C and in gel state after incubation for 1 hour at 15°C.

3. RESULT AND DISCUSSION

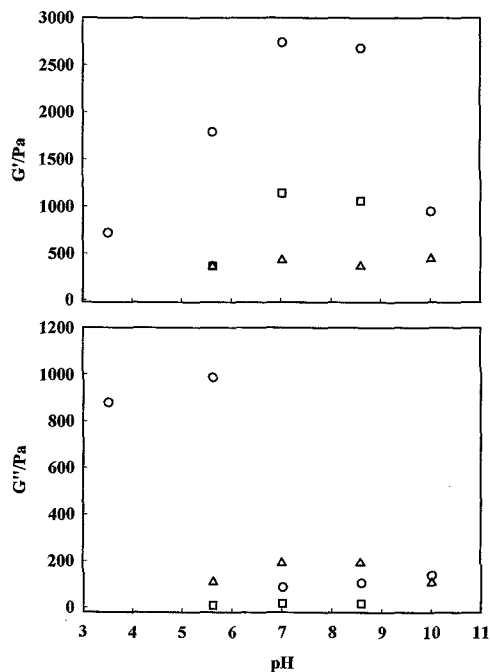


Fig. 1 pH dependence of storage modulus (G') and loss modulus (G'') at 3Hz for gelatin (\square), agar (\triangle) and mixture of gelatin and agar (\circ).

Figure 1 shows the pH dependences of G' and G'' for gelatin, agar and their mixture. It was found that G' and G'' for gelatin and the mixture were strongly affected by pH. However, there were no pH dependences of G' and G'' for agar except for pH 3.5. At the lower pH, 3.5, below gelatin's pI, the gel formation was observed only for the mixture except for gelatin and agar alone. Most of gelatin molecules are positively charged at the lower pH below its pI, indicating that the mixture forms a gel via the electrostatic interaction between positively charged gelatin and negatively charged agaropeptin which is the component of agar. At pH 5.6 very closed to gelatin's pI, the mixture formed a substantial gel with rather large G' , nevertheless the gelation of gelatin was distinctly inhibited. At intermediate pHs, 7.1 and 8.7, above the gelatin's pI, the mixture formed an elastic gel with large G' and small G'' . G' for the mixture was much larger than that for gelatin and agar alone. This result indicates that there exists a synergistic interaction between gelatin and agar. At higher pH, 10.1, far above the gelatin's pI, the mixture formed a gel with G' larger than that of agar even though gelatin alone did not form gel. It is considered that the mixture could form a gel composed of agar network reinforced by gelatin

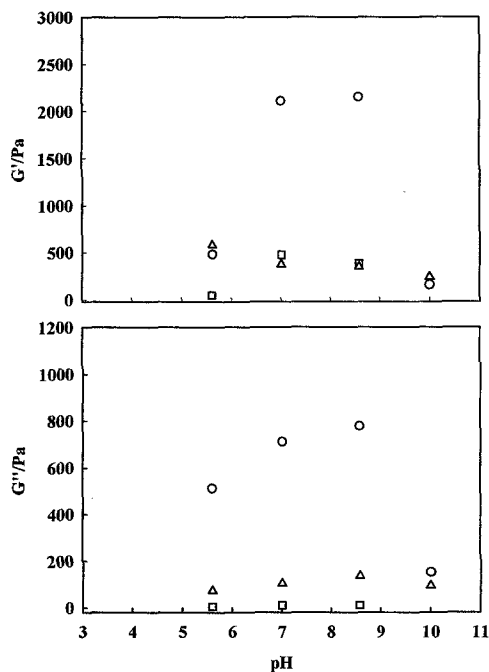


Fig. 2 pH dependence of storage modulus (G') and loss modulus (G'') at 3Hz for gelatin (\square), agar (\triangle) and mixture of gelatin and agar (\circ) in the presence of 0.5M NaCl.

molecules.

Figure 2 shows the pH dependences of G' and G'' for gelatin, agar and their mixture in the presence of 0.5M NaCl. G' and G'' for agar were scarcely affected by the addition of NaCl at all pHs. At pH 3.5, the gelation of the mixture was completely inhibited by the addition of NaCl. These results ensure that the mixture forms a gel via electrostatic interaction between positively charged gelatin and negatively charged agaropeptin. At pH 5.6, G' both for gelatin and the mixture considerably decreased even in the presence of 0.5M NaCl, indicating that there exists the electrostatic interaction between remaining positively charged gelatin and negatively charged agaropeptin. At intermediate pHs 7.1 and 8.7, G' for the mixture decreased, and G'' increased to the contrary. At higher pH 10.1, G' for the mixture decreased, and G'' was scarcely affected by the addition of NaCl. The decrease in G' could be brought about by the conformational change of gelatin and/or agar molecules at high ionic strength [9].

Figure 3 shows the pH dependence of loss tangent ($\tan\delta$) for gelatin, agar and their mixture in the absence (A) and the presence (B) of 0.5M NaCl. At pH 3.5, the mixture formed a very weak gel which dominated in viscous property with $\tan\delta$ larger than 1.

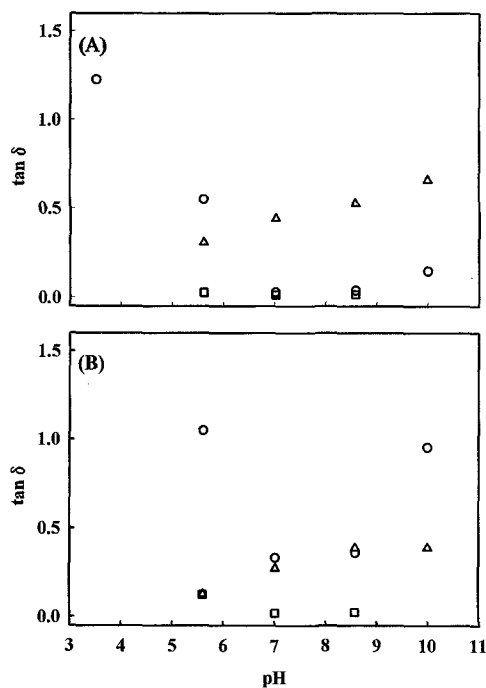


Fig. 3 pH dependence of loss tangent ($\tan\delta$) at 3Hz for gelatin (\square), agar (Δ) and mixture of gelatin and agar (\circ) in the absence (A) and the presence (B) of 0.5M NaCl.

At pH 5.6, $\tan\delta$ for the mixture was *ca.* 0.5 and increased to *ca.* 1 in the presence of 0.5M NaCl. At pHs 7.1, 8.7 and 10.0 above gelatin's pI, the mixture formed a strong and elastic gel with small $\tan\delta$. The addition of NaCl caused increase in $\tan\delta$ for the mixture but scarcely affected ones for gelatin and agar alone. It should be noted that ionic strength affects the viscoelastic properties of the mixture even above the gelatin's pI.

Figure 4 shows the pH dependence of τ for the mixture of gelatin and agar in sol and gel states in the absence (A) and the presence (B) of 0.5M NaCl. At pHs 3.5 and 5.6 not greater than gelatin's pI, τ for the mixture in sol state showed slightly high values, and then decreased in the presence of 0.5M NaCl. τ for the mixture was remarkably increased by the gelation except that transparent gel was formed at pH 3.5. At pH 5.6, τ for the mixture in gel state was distinctly decreased by the addition of NaCl. At pHs 7.1, 8.7 and 10.0 above gelatin's pI, opaque gels were formed with large τ which were not affected by the addition of NaCl. These results suggest that large τ could be caused by phase-separated networks due to demixing of the mixture prior to gelation [10].

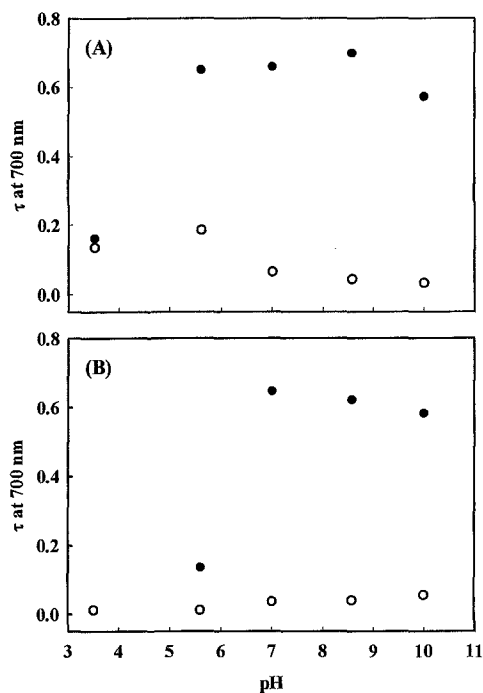


Fig. 4 pH dependence of turbidity (τ) mixture of gelatin and agar in sol (\circ) and gel (\bullet) states in the absence (A) and the presence (B) of 0.5M NaCl.

In this study, we measured the dynamic viscoelasticity and the turbidity of the mixture of gelatin and agar over a wide pH range in the absence and the presence of 0.5M NaCl. The results were summarized as follows:

- i) At lower pH 3.5 below gelatin's pI, the mixture formed a transparent and very weak gel with small τ and large $\tan\delta$, while, gelatin and agar alone did not form a gel. The gelation of the mixture was completely inhibited by the addition of NaCl, indicating that the mixture formed a gel via electrostatic interaction between poly-cationic gelatin and poly-anionic agarosectin.
- ii) At pH 5.6 very closed to gelatin's pI, the mixture formed an opaque and weak gel with large τ and large $\tan\delta$. Large τ could be caused by phase-separated networks due to demixing of the mixture prior to gelation. τ and $\tan\delta$ were considerably decreased and increased by the addition of NaCl, respectively. It is suggested that there exists the electrostatic interaction between remaining positively charged gelatin and negatively charged agarosectin.
- iii) At intermediate pHs 7.1 and 8.7 above gelatin's pI, the mixture formed an opaque and elastic gel with

large τ and small $\tan\delta$. G' for the mixture was much larger than that of gelatin and agar alone, indicating that there existed a synergistic interaction between gelatin and agar.

iv) At higher pH 10.1 far above gelatin's pI, the mixture formed gel with G' larger than that of agar even though gelatin did not form a gel. It is considered that the mixture could form a gel composed of agar network reinforced by gelatin molecules.

4. CONCLUSION

In conclusion, electrostatic interaction between gelatin and agar depends on the pH range, whether it is below or above the pI of gelatin. Synergistic interaction between gelatin and agar could be brought about by the formation of phase-separated networks due to demixing of the mixture prior to gelation. Ionic strength largely affects on the viscoelasticity and the turbidity for the mixture at pH not greater than gelatin's pI.

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