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Short Communications

Combination of temperature ramp with holographic relaxation spectroscopy for investigation of gelatin gels

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Abstract: A new method of combining temperature ramp with holographic relaxation spectroscopy (TR-HRS) to investigate thermoreversible gels is reported. The gel melting temperature, gel content, and mobility of the gelatin molecules inside the gel networks can be simultaneously measured in one TR-HRS experiment.

Key words: Holographic relaxation spectroscopy; temperature ramp; gelatin gels; thermoreversible gels

Introduction

Gelatin is a typical example of thermoreversible gels. When a sufficiently concentrated aqueous solution of gelatin is cooled down to a certain low temperature, the viscosity of the gelatin solution progressively increases in time and the liquid eventually turns into a gel without exception. If the temperature is increased beyond the gel melting temperature (T_m), the gel changes back to a solution. The setting of a gelatin solution, gel contents, gel strength, and T_m depend not only on the molecular structure, but also on the entire thermal as well as mechanical history. These physical parameters, the gel structures, and the kinetics of gelation are interesting to many researchers.

Various physical methods have been applied to investigate the structure of gelatin gels [1–3]. Some of them are just simply visual methods of observing the rising of air bubbles in the gel, flow ability of the solution, and other viscoelastic properties. These methods introduce some kind of mechanical perturbation to the gel, which affects the observation of the true nature of the system. Therefore, the measured characteristic parameters, such as the gel melting temperature, will depend on the shape and size of the gel sample. During the last ten years, a number of optical methods have been developed, which are based on measuring the mobility of sensor molecules inside polymer networks [4–14]. One such method, holographic relaxation spectroscopy (HRS), which is also known as forced Rayleigh scattering (FRS), was successfully applied to the gelatin gel by Chang and Yu [15]. Recently, Russo et al. [16] reported a temperature ramped fluorescence photo-bleaching recovery (TRFPR) technique for evaluating the gelatin gel. This technique is an extension of the fluorescence photobleaching recovery (FPR) technique.

In this discussion, by using gelatin as one example, we will introduce another mobility-based optical method for characterizing thermoreversible gels. Our method combines temperature ramp with holographic relaxation spectroscopy (TR-HRS), which is an extension of HRS technique. It has principles similar to the TRFPR technique. The gelatin molecule is covalently labeled with a small photochromic molecule: fluorescein-isothiocyanate. After gelation, some of the labeled gelatin molecules form a gel network ("gel-phase") and the rest remain in the sol ("sol-phase"). Therefore, mobility of the labeled gelatin molecules inside the

gel network and the gel network itself are probed. By measuring the intensity relaxations both before the temperature ramp and after, the translational diffusion coefficient (D) of gelatin molecules in the sol, gel content, and T_m can be simultaneously obtained in one experiment, which typically takes $\frac{1}{2}$ h, without introducing any mechanical or chemical perturbations. Preliminary measurements on the gelatin gels are reported. The results are mainly used to demonstrate how TR-HRS can be applied to evaluate thermoreversible gels. In principle, TR-HRS can be used to investigate other thermoreversible gels as long as the polymer in the gels can be covalently labeled with a photochromic probe, i.e., if the polymer does not have intrinsic photochromic properties. Furthermore, the principle of TR-HRS could be used to develop other ramped HRS methods, such as pressure ramped HRS and electric field ramped HRS for evaluating other reversible gels.

We should point out that TR-HRS has several advantages compared with TRFPR: i) higher signal/noise ratio, because the measured relaxation intensity is from a diffracted laser beam and the background noise can be easily reduced to a negligible level by an interference filter; ii) well-defined baseline for calculating the gel content; iii) no disturbance during the measurements; iv) well-defined T_m; and v) higher sensitivity in measuring the gel content especially when the gel network only contains small amounts of sol, which is the case for most of the interesting gelatin gels. All these aspects will be discussed fully.

Experimental part

Sample preparation

Gelatin used in this experiment was purchased from Deutsche Gelatine-Fabriken Stoess. It is a pharmaceutical-grade B-type gelatin (Bloom value 200), i.e., it was obtained from alkaline-treated bone stock. The GPC measurements (courtesy of Dr. Klaus Bräumer and Dr. Wilfried Babel, Deutsche Gelatine-Fabriken Stoess, Eberbach) shows that the weight-averaged molecular weight (M_w) and the number-averaged molecular weight (M_n) are 1.4×10^5 g/mol and 6.3×10^4 g/mol, respectively. Fluorescein-isothiocyanate was from Serva. Both of them were used without further purification. The gelatin solutions were prepared by first dissolving a proper amount of gelatin in a buffer solution (pH = 10, $Na_2B_4O_7$/NaOH) at 50 °C for at least 1 h to form a uniform gelatin solution, then adding fluorescein-isothiocyanate to the solution. The solution was kept at the same temperature with very mild stirring for about 1 h. Mol ratio of fluorescein-isothiocyanate to gelatin was less than 1.0. The lack of free fluorescein-isothiocyanate was justified in this preparation method [15]. After labeling the gelatin, the solution was placed in a Hellma-made rectangle cell with 1-mm path length, then it was cooled down to 25 °C at a rate of 2 °C/min and matured for at least 17 h before the measurements. Each time, five samples were prepared and measured in the same way in order to check the reproducibility of the measurements.

TR-HRS experiment

Details of HRS experiment can be found elsewhere [5]. For the convenience of discussion, we only outline the basic principle of HRS. By crossing two coherent laser beams (writing beams) on the same spot in the sample for a very short time (20 ms for all experiments reported in this study), a spatial modulation of refractive index and/or absorption coefficient, i.e., an optical grating, is artificially created via a photochromic reaction. After this writing pulse, the periodic concentration distributions of the reacted and unreacted molecules are gradually be smeared out by the translational diffusion. This relaxation process can be directly followed by measuring the transient diffraction intensity with another laser beam (reading beam) at Bragg's angle passing through the same spot previously illuminated by the writing laser beam.

Figure 1 schematically shows our TR-HRS set up. Basically, it is an HRS instrument combined with a temperature ramp controller (Huber PD 420) and a temperature recorder (BBC SE 120), which is synchronized with the HRS measurement. A needle-type thermometer (AMR 2210-7) is directly placed inside the gel sample near the measuring spot to monitor the temperature. A 400 mW Argon ion laser from Coherent (INNOVA 90) operated at 488 nm is used to produce the writing beams. The sample temperature from about 5 °C to 80 °C with a precision of $\pm$ 0.1 °C can be controlled by a home-made cell holder with a thermostat. The upper limit of the temperature ramp rate is 4 °C/min due to the heating capacity of the thermostat, and in principle there is no lower limit for the rate. The fringe spacing can be varied from 0.8 to 40 μm by changing the crossing angle between two writing beams. Because the reading beam is far away from the absorption band of fluorescein-isothiocyanate, the diffraction intensity is mainly from the phase change, i.e., "phase HRS." Therefore, the reading beam can monitor the relaxation process without any heating or introducing further photochromatic reactions. In contrast, the probe molecules in TR-FPR are continuously bleached by the reading beam during the measurements, which disturbs the measurement.

Results and discussion

Figure 2 shows a typical initial part of an HRS spectrum for a 6.67% solution of the gelatin in the buffer measured at 25 °C, which is below T_m ($\sim$ 31.8 °C). In general, the relaxation process measured by the output signal $I(V)$ of a photo-

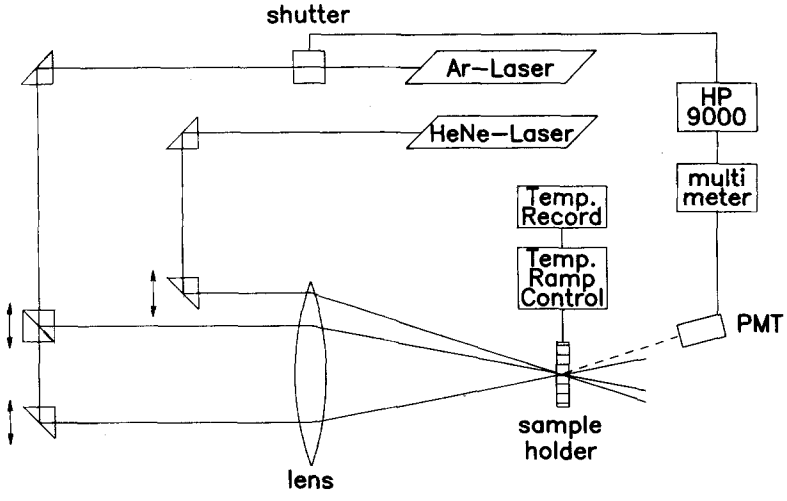

Fig. 1. Schematic diagram of our TR-HRS set up. Basically, it is a combination of HRS with a synchronized temperature ramp. A 36.6-μm fringe spacing and a 20-ms writing pulse generated by the shutter were used in this study

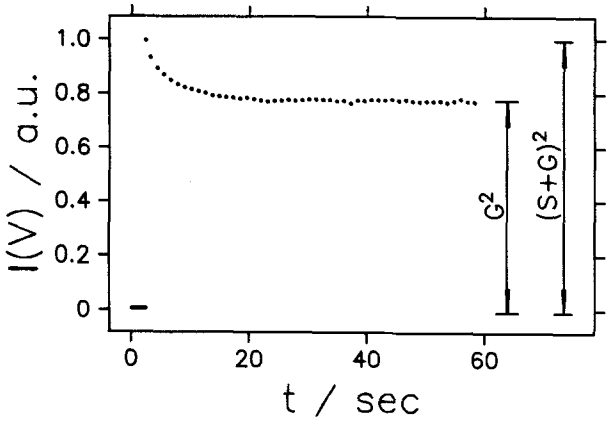

Fig. 2. Typical initial part of TR-HRS spectrum before the temperature ramp for a 6.67% solution of the gelatin in the buffer. The sample was matured and measured at 25 °C. The gel content calculated from $G/(S+G)$ is (88 ± 2)% and the averaged translational diffusion coefficient $\langle D \rangle$ from a second order cumulants fitting is $(7.5 \pm 0.5) \times 10^{-8}$ cm^2 sec^{-1}

multiplier can be represented by

$$I(V) = [s(t) + g(t) + B]^2 + C^2 , \qquad (1)$$

where $s(t)$ and $g(t)$ denote the respective electric field of the diffracted light from the "sol phase" and the "gel phase." B and C are for the coherent and the incoherent background scattering, respectively. $s(t)$ can be further expressed as $\sum_i S_i \exp(-D_i K^2 t)$, where D_i and S_i are the translational diffusion coefficient and the electric field of the diffracted light of the ith component of the gelatin molecules in the sol, respectively, and K $(= 2\pi/L)$ is a constant at a fixed fringe spacing (L) of the optical grating. Below T_m, $g(t)(= G)$ is a constant, because the gelatin molecules in the "gel phase" are immobile. In our experiment, B and C can be taken as zero because they are so small in comparison with $s(t)$ and $g(t)$. So, when $T < T_m$, Eq. (1) can be reduced to

$$I(V) = [\textstyle\sum_i S_i \exp(-D_i K^2 t) + G]^2 . \qquad (2)$$

Based on Eq. (2), $I(V) = (S+G)^2$ when $t = 0$, where $S = \sum_i S_i$, and $I(V) = G^2$ when $t \gg (D_i K^2)^{-1}$. Both $(S+G)^2$ and G^2 are schematically shown in Fig. 2. From a coupled wave theory [17] for a loss-less transmission grating without slant, S and G are proportional to the amplitudes of the spatial modulation of the probe concentration as long as the probe concentration is very dilute ($\sim 10^{-4}$ g/ml in this study). The gelatin molecules are assumed to be evenly labeled with the probe molecules. Therefore, the probe concentration should be further proportional to the gelatin concentration. In this way, Eq. (2) can be rewritten as

$$I(V) = p(C_s + C_g)^2 \quad \text{when } t = 0 \qquad (3)$$

and

$$I(V) = pC_g^2 \quad \text{when } t \gg (D_i K^2)^{-1} , \qquad (4)$$

where p is simply an overall proportional constant and C_s and C_g are the concentrations of the gelatin molecules in the "sol phase" and in the "gel phase," respectively. Experimentally, the gel content can be measured from the square root of $G^2/(S+G)^2$. In comparison with TRFPR, where the measured fluorescence intensity is proportional to the concentration, TR-HRS is more sensitive to samples containing a small amount of sol, because

the measured diffraction itensity is proportional to the square of the concentration. For example, a sample containing 10% sol (i.e., 90% gel) will give about 20% relaxation in the intensity. The measured gel content for the sample in Fig. 2 is (88 ± 2)%. The uncertainty is mostly from five repeat measurements.

The averaged translational diffusion coefficient ($\langle D \rangle$) of the gelatin molecules in the sol can be calculated from the relaxation before the temperature ramp by using a cumulants method. In Fig. 2, $\langle D \rangle = (7.5 \pm 0.5) \times 10^{-12}\ m^2\ sec^{-1}$. A Laplace transform method, such as CONTIN [18–20] or MSVD [21], can be applied for a detailed analysis of the initial relaxation. The Laplace transform can give each D_i and S_i. They should be very useful for interpreting the structure of gel networks. Unfortunately, a more detailed discussion cannot be justified at this moment because of limited results.

In order to determine T_m, the temperature ramp was performed when the initial relaxation was finished. Figure 3 shows a typical complete TR-HRS spectrum with 1 °C/min ramp speed for a 15 wt.% solution of the gelatin in the buffer. The sample was matured and measured at 25 °C. In an ideal situation, the intensity after the ramp should first stay constant until the temperature reaches to T_m and then decrease after T_m, which is presented by the dot-line in Fig. 3. In contrast to the ideal intensity profile, the intensity first increases remarkably as the temperature rises and then drops sharply after a certain temperature. This turning point is experimentally defined to be T_m, which was cross-checked by a visual method.[1] In Fig. 3, T_m measured by TR-HRS is 32.6 ± 0.1 °C and T_m measured by the visual method is 32.3 ± 0.5 °C. The uncertainties are also from five repeat measurements.

In order to understand this unexpected intensity increase and check the possible inhomogeneous effect or possible change of miscibility, the scattered intensity from a sample without introducing the optical grating was monitored during the heating process. It was found that the scattering intensity is not very sensitive to the temperature. Furthermore, two other samples were prepared: one by adding 0.1 M NaCl to the buffer solution and the other by adding distilled water to the buffer solution (1:1 by weight). Both samples also showed an unexpected similar intensity increase, which means that the increase is not related to the ionic strength of the solution.

In order to further understand this unexpected increase in the intensity, a set of samples was prepared and measured in the same way, except the final measurement temperatures were different. Figure 4 shows a plot of the measured initial diffraction intensity $I(t = 0)$ vs the experimental temperature for a 15% solution of gelatin in the buffer. It suggests that the diffraction efficiency of the optical grating changes with the temperature, because other experimental conditions are identical.

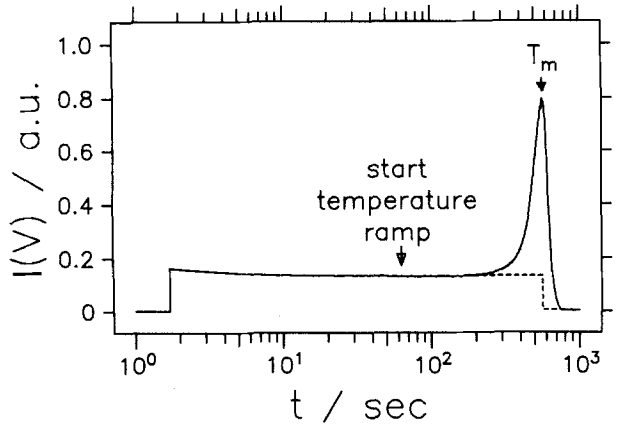

Fig. 3. Typical complete TR-HRS spectrum for a 15% solution of gelatin in the buffer. The sample was matured and measured at 25 °C. The temperature ramp rate is 1 °C/min. The measured T_m is 32.6 ± 0.1 °C

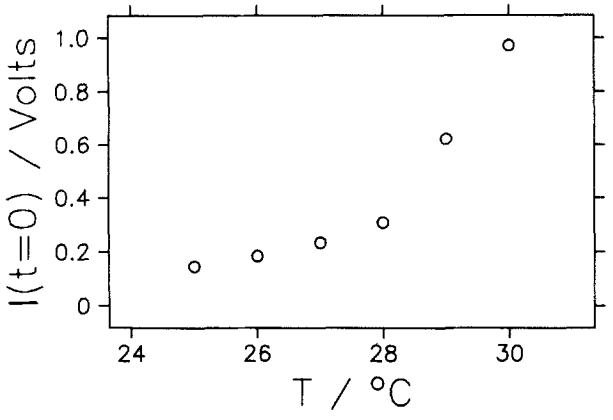

Fig. 4. Plot of initial diffraction intensity $I(t = 0)$ vs the experimental temperature (T) for a 15% solution of gelatin in the buffer. All samples were prepared and measured in an identical procedure, except the final measurement temperatures were different

The higher the temperature, the higher the diffraction efficiency will be. The physical background for the change is still unknown.

By using TR-HRS, it is found that the gel content exponentially increases with the gelatin concentration and the setting time. It is also found that the gel content is proportional to the reciprocal of the setting temperature. The measured T_m increase with the gelatin concentration and the measured $\langle D \rangle$ is almost independent of the gelatin concentration as long as the concentration is larger than 5%. Further data analysis and discussion of the structure and dynamics of gelatin gels will be presented separately in a following publication.

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