# Application of the Temperature-Ramped Holographic Relaxation Spectroscopy in the Investigation of Physically Cross-Linked Gels

**Chengqing Wang, Suhong Jiang, and Chi Wu***

*The Open Laboratory of Bond-selective Chemistry, Department of Chemical Physics, University of Science & Technology of China, Hefei, Anhui, China, and Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong*

*Received March 12, 2001*

ABSTRACT: Holographic relaxation spectroscopy (HRS), also known as forced Rayleigh scattering (FRS), can be used to study slow translational diffusion of probes inside a polymer gel. In this study, we demonstrated that, besides the translational diffusion coefficient, a combination of temperature ramp with HRS could lead to additional information on physically cross-linked gels, such as the gel content, the gel melting temperature, and the gel structure. Using a gelatin gel as an example, we correlated the temperature dependence of the intensity of the diffracted light to the formation of triple-stranded helixes (i.e., the renaturalization process). Such a correlation was supported by the optical rotation study. Our results showed that the renaturalization followed the first-order kinetics. The applications of such a combination of the temperature ramp with HRS have been envisioned.

## Introduction

In general, polymer gels can be classified as chemical gels or physical gels, depending on whether the cross-linking between polymer chains is formed via a covalent bond or physical interaction, such as hydrogen bonding and complexation. The sol−gel ratio of a given gel is an important parameter because it is directly related to the gel strength. For a chemical gel, one can use a good solvent to extract the sol portion and measure the sol−gel ratio by a conventional gravimeter. But for a physical gel, the determination of the sol−gel ratio has been a remaining challenge because the measurement could easily disturb the weak cross-linking points to make the result less reliable. On the other hand, the melting temperature of a physical gel is also an important parameter. It is normally determined by a macroscopic flowing test which is a viscoelastic test because the gravity force is applied.

A number of optical methods have recently been developed to measure the mobility of molecular sensors inside a polymer gel network. One of such methods is holographic relaxation spectroscopy (HRS), also known as forced Rayleigh scattering (FRS). In 1984, Chang and Yu[1] successfully used it to study the mobility of labeled gelatin chains inside a gelatin gel, and the results were comparable to those from dynamic light scattering. It has also been shown that the diffusion of various probes inside the gelatin network studied by HRS could lead to some additional information.[2,3]

The gelatin gel is a typical physical gel. It can undergo a thermoreversible gelation if the gelatin concentration is higher than 2−3 wt %.[4−6] Most of the past studies were focused on the investigations of its conformation change in solution, the sol−gel transition, and its rheological properties because it is one of the most abundantly found proteins in mammals and also because it has various industrial applications.[7−24] It is generally known that the sol−gel transition involves a renaturalization process; namely, the gelatin chains form fibrous triple-stranded helixes, and the aggregation of these helixes leads to small crystal junctions which act as cross-linking points and build up a three-dimensional gel network. However, the exact conformational path has not been conclusively established yet.

Previously, using the gelatin as an example of physically cross-linked gels, we demonstrated that a combination of the temperature ramp and HRS could also be used to determine the melting temperature and the gel content of physical gels.[23,24] In that study, we observed an unexpected increase of the intensity of the diffracted light near the gel melting temperature. In the present study, we investigated why there existed such an abnormal intensity increase and whether a combination of the temperature ramp and HRS could be used to monitor the structure change of physical gels.

## Experimental Section

**Sample Preparation.** Pharmaceutical-grade gelatin (Bloom value 270, Courtesy of Deutsche Gelatine-Fabriken Stoess) was prepared from the alkaline-treated bone stock. The light-scattering characterization showed that it had a weight-averaged molar mass ($M_w$) of $3.0 \times 10^5$ g/mol.[25] The gelatin solutions were prepared by dissolving a proper amount of gelatin in a buffer solution (pH = 10, $H_3BO_3$/KCl/NaOH) at 50 °C. The gelatin was labeled by fluorescein−isothiocyanate in solution. All the fluorescein−isothiocyanate molecules were chemically bonded to the gelatin chains.[1] On average, each gelatin chain had no more than one fluorescein−isothiocyanate molecule. It was reasonable to assume that the labeling had no effect on the chain thermodynamics. The hot solution was injected into a disk-shaped cell (15 mm in diameter and 1 mm in thickness) and gelled at a desired temperature below 30 °C. The cooling was controlled at a rate 2 °C/min. Before the HRS measurement, each gel was matured at the gelling temperature for at least 2 days.

**TR-HRS.** As shown in Figure 1, it combines an HRS instrument with a temperature ramping device. The reading beam was vertically polarized to the paper. By moving the detector to zero angle and placing a 90° polarizer in front of it, we were able to measure the depolarization of the transmitted light. The basic principle of TR-HRS is as follows. By crossing two coherent laser beams (the writing light, $\lambda = 488$ nm, Coherent Innova 90 $Ar^+$ laser, 400 mW) within a sample,

* The Hong Kong address should be used for correspondence.

10.1021/ma010436e CCC: $20.00 
Published on Web 08/15/2001

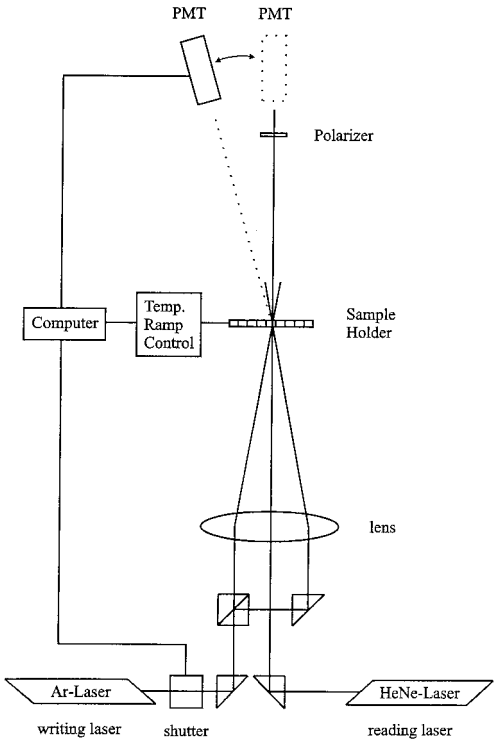


**Figure 1.** Schematic of a combination of holographic relaxation spectrometer (HRS) with a temperature ramp device.

which contains photochromic probes, for a short time (typically 20 ms), one can create a spatial modulation of refractive index and/or absorption coefficient, (i.e., an optical holographic grating) via a photochromic reaction. After the writing pulse, the translational diffusion of the probes smears the periodic concentration distribution made of the photochromically reacted and unreacted probes. This relaxation can be directly monitored by recording the intensity of the diffracted second laser beam (the reading beam, $\lambda = 632$ nm, Uniphase HeNe laser, 10 mW) passing through the grating. Note that we have chosen a normal incidence of the reading beam instead of a more efficient Bragg incidence. The advantage was that we did not need to adjust the reading beam each time after we alternated the crossing angle between the two writing beams. With a 10 mW HeNe laser, the lower diffraction efficiency was not a problem.

In this study, the wavelength of the reading beam was so far away from the absorption band of fluorescein−isothiocyanate that the diffraction was mainly caused by refractive index difference between the reacted and unreacted probes inside the grating. The translational diffusion of free chains in the sol can smear part of the grating, while the chains attached to the gel network are immobile, preventing a complete relaxation of the diffraction intensity. The extent of the relaxation can be related to the gel content.[4]

## Results and Discussion

Figure 2 shows a typical TR-HRS signal of the fluorescein−isothiocyanate labeled gelatin gel. The slight relaxation in the initial stage before the temperature ramp started was related to the translational diffusion of a small amount of free gelatin chains in the sol. When the temperature was ramped higher than the melting temperature ($T_m$), the immobile gel network broke into small mobile fragments (individual chains and clusters) so that the second relaxation occurred. In principle, the diffraction intensity [$I(t)$] should remain a constant until the temperature reaches $T_m$, i.e., following the dotted line. However, Figure 2 shows an unexpected increase of $I(t)$ as the temperature approached $T_m$. The temperature was measured by a small flat thin thermistor with a time constant ~0.6 s. Our previous study showed that such an increase of $I(t)$ was not due to small-angle scattering related to the inhomogeneity or fluctuation near the melting temperature.[4]

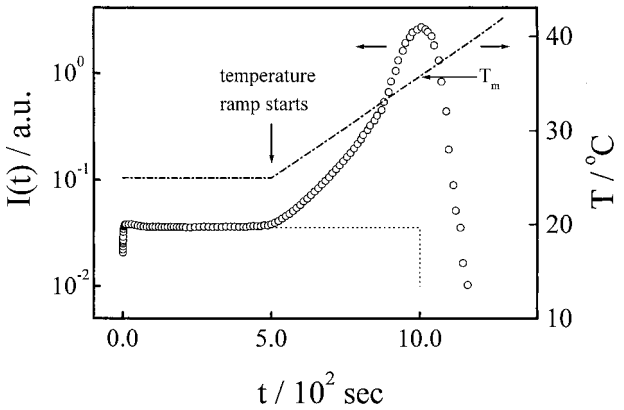


**Figure 2.** Typical TR-HRS spectrum of a gelatin gel, where $C = 13$ wt % and pH = 10.

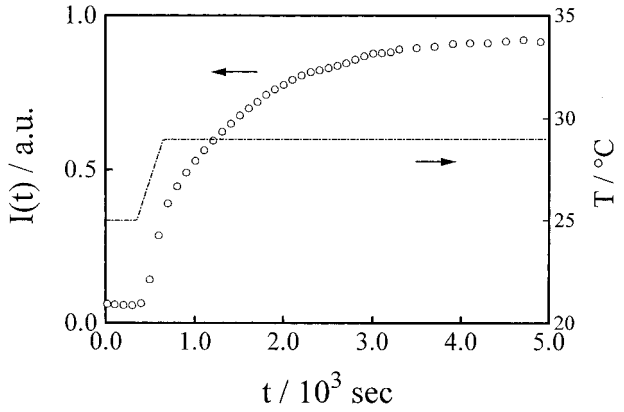


**Figure 3.** Temperature dependence of the diffracted light intensity, where the gelatin gel was matured at 25 °C for 2 days before the measurement.

Figure 3 shows the TR-HRS spectrum of the gel containing 13 wt % gelatin, where the gel was matured and photobleached at 25 °C. The initial decay associated with the translational diffusion is not shown since here we are only interested in the increase of $I(t)$ with the temperature near the melting temperature $T_m$ in the range 25−29 °C. Note that even after the temperature ramp was stopped at 29 °C, $I(t)$ still increased and finally approached a constant after 1.5 h, much longer than the ramping time (~5 min) required to reach the measuring temperature. It implied that the gel structure changed differently within the bleached and unbleached stripes at temperatures higher than the gelling temperature. We also found that after the gel was cooled to 25 °C, $I(t)$ did not return to the initial value before the temperature ramp. This indicates that the structure difference between the bleached and unbleached stripes formed at higher temperatures remained.

A long time ago, the X-ray diffraction pattern revealed the formation of very small locally ordered crystallites when a gelatin gel was matured at a temperature near the melting temperature.[26] Also note that the bleached stripes were heated and quenched after the writing pulse. Our previous study showed that more fibrous triple-stranded helixes were formed if the gelatin solution was very slowly cooled below the gelation temperature, while a quick quenching froze the gelatin chains

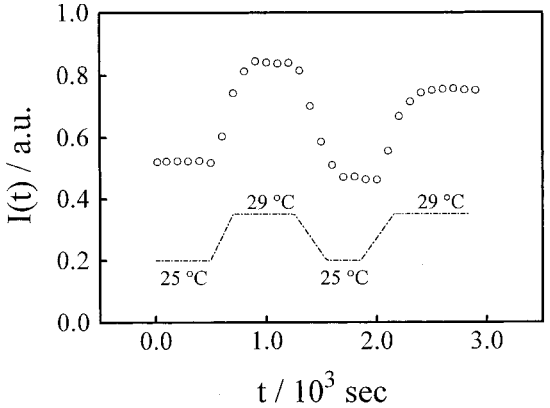


**Figure 4.** Temperature dependence of the diffracted light intensity, where the gelatin gel was matured at 29 °C for 2 days before the measurement.

into the amorphous state.[4] As expected, the gelatin chains at higher temperatures near $T_m$ are more mobile. The increase of $I(t)$ with the temperature near $T_m$ suggested that the structure change was different between the bleached and unbleached stripes, which affected the contrast of the optical grating. Such a structure change must involve a dimension smaller than the grating width (8.16 $\mu$m in the present HRS experiment). Otherwise, we would observe a decrease in $I(t)$.

Figure 4 shows that if the gel was gelled and matured at 29 °C instead of 25 °C, the increase of $I(t)$ in an identical temperature ramp range was much less than that in Figure 3. The increase of $I(t)$ ceases nearly as soon as the temperature ramp stopped. This is expected because the gel was matured at 29 °C, and no additional structure could be further formed. Note that $I(t)$ follows the temperature change, clearly indicating that the diffraction efficiency of the grating was dependent on the temperature. A combination of Figures 3 and 4 shows that only part of the unexpected increase of $I(t)$ was related to the formation of additional structures, presumably, the triple-stranded helixes. Also note that a similar increase of the diffraction intensity was also observed when the temperature approached the nematic−isotropic transition temperature in *n*CB's ($n = 5-9$) and *N*-(4-methoxybenzylidene-4-*n*-butylaniline) (MBBA) liquid crystals.[27,28] In the case of *n*CB, the anomalous increase of the diffraction intensity was interpreted as a photostimulated change in the phase transition temperature.[29] While for MBBA, the observed temperature dependence of the diffraction intensity was attributed to the variation of the order parameter; i.e., the fluctuation of the order parameter became larger as the temperature approached to the phase transition.

In general, $I(t) = [s(t) + g(t) + B]^2 + C^2$, where $s(t)$ and $g(t)$ respectively denote the electric fields of the diffracted lights from the sol and the gel; $B$ and $C$ are respectively the electric fields of coherent and incoherent scattered lights (backgrounds). In our setup, both $B$ and $C$ were negligible in comparison with $s(t)$ and $g(t)$. Hence, the equation is simplified as $I(t) = [s(t) + g(t)]^2$. When the temperature ramp starts, $s(t)$ already completely relaxed, so that $I(t) = [g(t)]^2$. If some additional structures were formed at higher temperatures, $g(t)$ would contain the contributions from both the old and new structures, i.e., $g(t) = g_{old}(t) + g_{new}(t)$.

On the basis of the coupled wave theory,[30] for a lossless transmission grating without slant, $g_{old}(t)$ and $g_{new}(t)$ are proportional to the amplitude of spatial

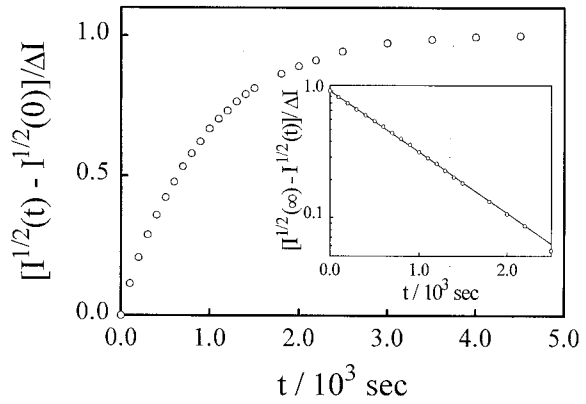


**Figure 5.** Time dependence of the relative diffracted light intensity, $[I^{1/2}(t) - I^{1/2}(0)]/\Delta I$, after the gel temperature was ramped to 29 °C, where $I(0)$, $I(t)$ and $I(\infty)$ are the diffracted intensities at time zero, $t$, and infinity, respectively, and $\Delta I = I^{1/2}(\infty) - I^{1/2}(0)$. The line is a least-squares fitting of $[I^{1/2}(\infty) - I^{1/2}(t)]/\Delta I = \exp(-1.05 \times 10^{-3}t)$.

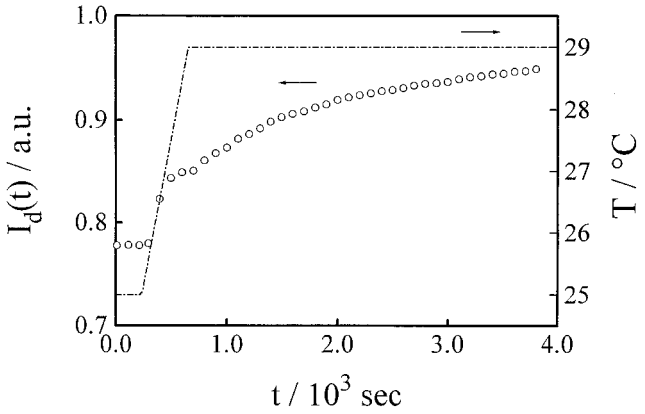


**Figure 6.** Temperature dependence of the depolarized light intensity, where the gelatin gel was matured at 25 °C for 2 days before the measurement.

concentration modulation as long as the concentration is dilute. Therefore, $g(t) = P_{old}N_{old}(t) + P_{new}N_{new}(t)$, where $N_{old}(t)$ and $N_{new}(t)$ are the concentrations of old and new structures, respectively, and $P_{old}$ and $P_{new}$ are two constants respectively related to the diffraction efficiencies of old and new structures. The total concentration ($N$) of the triple helixes in the gel network is the sum of $N_{old}$ and $N_{new}$, i.e., $N = N_{old} + N_{new}$. Therefore, $I(t) = [P_{old}N + (P_{new} - P_{old})N_{new}(t)]^2 = [A + BN_{new}(t)]^2$ or written as $I^{1/2}(t) = A + BN_{new}(t)$, where $A = P_{old}N$ and $B = (P_{new} - P_{old})$. $I^{1/2}(t)$ is proportional to the concentration of the new triple helixes. As shown in Figure 5, the normalized relative intensity ($[I^{1/2}(\infty) - I^{1/2}(t)]/\Delta I$) follows a single-exponential decay, i.e., $[I^{1/2}(\infty) - I^{1/2}(t)]/\Delta I = \exp(-kt)$, where $I(0)$, $I(t)$, and $I(\infty)$ are the diffraction intensities at time zero, $t$ ,and infinity, respectively; $k$ is a rate constant, and $\Delta I \equiv I^{1/2}(\infty) - I^{1/2}(0)$. We have assumed that all the new structures were formed at 29 °C because the ramping time is relatively short. It clearly shows that the formation of the new structures at 29 °C follows the first-order kinetics.

Figures 6 and 7 reveal that the depolarized and diffracted light intensities have a similar temperature dependence. When the gel was gelled and matured at 25 °C, the depolarized light intensity increased significantly when the temperature ramped from 25 to 29 °C and continued to increase even after the temperature ramp was stopped at 29 °C. In contrast, when the gel

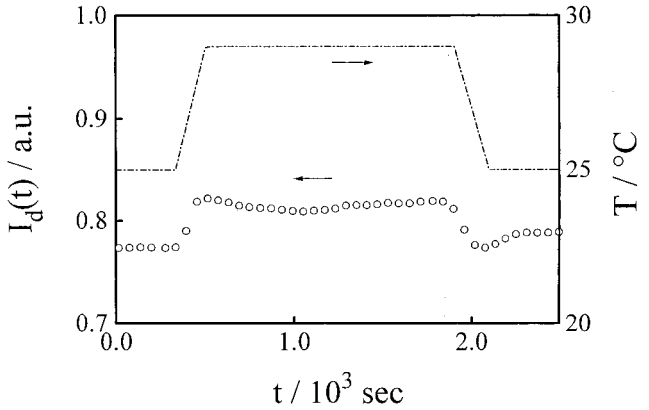


**Figure 7.** Temperature dependence of the depolarized light intensity, where the gelatin gel had been matured at 29 °C for 2 days before the measurement.

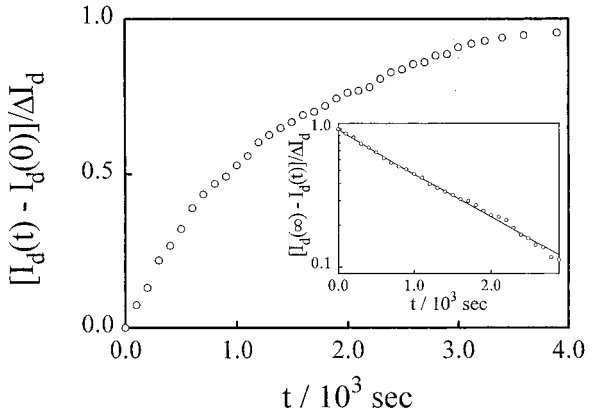


**Figure 8.** Time dependence of $[I_d(t) - I_d(0)]/\Delta I_d$ after the gel temperature was ramped from 25 to 29 °C, where $I_d(0)$, $I_d(t)$, and $I_d(\infty)$ are the depolarized light intensity at time zero, $t$, and infinity, respectively, and $\Delta I_d = I_d(\infty) - I_d(0)$.

was matured at 29 °C, the increase of the depolarized light intensity with the temperature was much less and stopped as soon as the temperature ramp was finished. It is known that the formation of the triple helixes in the gelatin renaturalization process can increase its optical rotation. The depolarized light intensity $[I_d(t)]$ can be related to the optical rotation by $I_d(t) = I_0 \sin \alpha + C$, where $I_0$ is the incident light intensity, $\alpha$ is the optical rotation angle, and $C$ is a constant related to the extinguish ratio of the polarizer. For a thin cell with an optical pass length of 1 mm, $\alpha$ from the gelatin gel is normally so small that $I_d(t) \approx I_0\alpha + C$. The increase of the depolarized light intensity could be analyzed by a similar equation used in Figure 5, i.e., $[I_d(\infty) - I_d(t)]/\Delta I_d = \exp(-k't)$, where $I_d(0)$, $I_d(t)$, and $I_d(\infty)$ are the depolarized light intensities at time zero, $t$, and infinity, respectively; $k'$ is a rate constant, and $\Delta I_d = I_d(\infty) - I_d(0)$.

Figure 8 shows such a plot on the basis of Figure 6, where $k' = 8.8 \times 10^{-4}$ s$^{-1}$. It further indicates that the formation of additional helixes followed the first-order kinetics. Note that $k$ and $k'$ are fairly close. It has been known for a long time that the gelatin renaturalization consists of three processes: nucleation, triple-helix formation, and annealing.[9] Each of them follows the first-order kinetics. The nucleation is not favorable at the temperatures near $T_m$, and the annealing requires a time scale much longer than that used in the present study. Therefore, we have observed the formation of additional triple-stranded helixes at 29 °C.

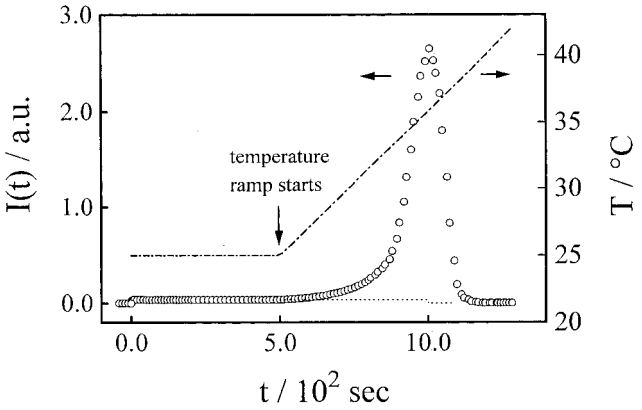


**Figure 9.** Maturing time dependence of the diffracted light intensity just after the photobleaching, where the gel matured and measured at 25 °C.

To confirm it, we measured the initial diffraction intensity after maturing the gel at 25 °C for different times. It was expected that a longer maturing time would result in more helixes. Figure 9 confirms this speculation. It should be noted that the gelatin chains in the triple helixes are more closely packed and ordered than those in the amorphous state. Therefore, the bleaching may enhance the difference in the optical property, i.e., leading to a higher contrast between the reacted and nonreacted probes inside the triple helixes. Further, our results showed that the diffracted light did not contain the depolarized component. Therefore, the intensity increase of the diffracted light was certainly not due to the change of the optical rotation power but related to the structure difference between the bleached and unbleached stripes formed at higher temperatures.

## Conclusion

We compared the gelling temperature dependence of the diffracted and depolarized light intensities of the gelatin gel near its melting temperature in the temperature-ramp holographic relaxation spectroscopy (TR-HRS). Our results showed that there existed an unexpected increase of the diffraction intensity near the melting temperature. It could be partially related to both the formation of some additional triple helixes, presumably in the unbleached stripes at higher temperatures. Our results demonstrated that by monitoring the change of the diffraction intensity, one could use the TR-HRS to monitor the structure change of a physical gel. In comparison with other conventinal methods, we can use the TR-HRS to study a structure change smaller than a few micrometers with a much higher signal-to-noise ratio and a much less amount of sample, e.g., ~10 mg in comparison with ~1 g used in a normal optical rotation measurement. Such temperature-dependent diffraction in the TR-HRS can be envisioned in other studies, such as the crystallization of polymers; namely, we can use the writing beam to induce or melt polymer crystallites in the stripes and study the crystallization by monitoring the intensity of the diffracted reading beam.

**Acknowledgment.** The financial support of the CAS Bai Ren Project, the NNSFC projector (29974027), and the HKSAR Earmarked RGC Grants (CUHK/4266/00P, 2160135) is gratefully acknowledged.

## References and Notes


(1) Change, T.; Yu, H. *Macromolecules* **1984**, *17*, 115.
(2) Yoon, H.; Kim, H.; Yu, H. *Macromolecules* **1989**, *22*, 848.
(3) Wu, C.; Schrof, W.; Lilge, D.; Luddecke, E.; Horn, D. *Makromol. Chem., Macromol. Symp.* **1990**, *40*, 153.
(4) Wu, C.; Schrof, W.; Luddecke, E.; Horn. D. *Colloid Polym. Sci.* **1991**, *269*, 523.
(5) Flory, P. J. *J. Phys. Chem.* **1942**, *46*, 132.
(6) Ferry, J. D. *Adv. Protein Chem.* **1948**, *4*, 1.
(7) Clark, A. H.; Ross-Murphy, S. B. *Adv. Polym. Sci.* **1987**, *83*, 57.
(8) Veis, A. *The Macromolecular Chemistry of Gelatin*; Academic Press: London, 1964.
(9) Harrington, W. F.; Karr, G. M. *Biochemistry* **1970**, *9*, 3725.
(10) Borchard, W.; Bergmann, K.; Emberger, A.; Rehage, G. *Prog. Colloid Polym. Sci.* **1976**, *60*, 120.
(11) Ward, A. G.; Courts, A. *The Science and Technology of Gelatin*; Academic Press: London, 1977.
(12) Djabourov, M.; Papon, P. *Polymer* **1983**, *24*, 537.
(13) Newcomer, M. E.; Jones, T. A.; Aqvist, J.; Sundelin, J.; Eriksson, U.; Rask, L.; Peterson, P. A. *EMBO J.* **1984**, *3*, 1451.
(14) Borchard, W.; Luft., B.; Reutner, P. *J. Photogr. Sci.* **1986**, *34*, 132.
(15) Russo, P. S.; Mustafa, M.; Tipton, D.; Nelson, J.; Fontenot, D. *Polym. Mater. Sci. Eng.* **1988**, *59*, 605.
(16) Djabourov, M. *Contemp. Phys.* **1988**, *29*, 273.
(17) Yoon, H.; Kim, H.; Yu, H. *Macromolecules* **1989**, *22*, 848.
(18) Quellet, C.; Eicke, H. F.; Gehrke, R.; Sager, W. *Europhys. Lett.* **1989**, *9*, 293.
(19) Takahashi, H.; Choi, P. K. *Jpn. J. Appl. Phys., Part 1* **1996**, *35*, 2939.
(20) Tsukida, N.; Maeda, Y.; Kitano, H. *Macromol. Chem. Phys.* **1996**, *197*, 1681.
(21) Huang, H.; Sorensen, C. M. *Phys. Rev. E* **1996**, *53*, 5075.
(22) Yokoyama, C.; Tamura, Y.; Takahashi, S.; Takeuchi, K. *Fluid Phase Equilib.* **1996**, *117*, 107.
(23) Michon, C.; Cuvelier, G.; Relkin, P.; Launay, B. *Int. J. Biol. Macromol.* **1997**, *20*, 259.
(24) Xia, J.; Wang, C. H. *Macromolecules* **1999**, *32*, 5655.
(25) Wu, C. *J. Polym. Sci., Part B: Polym. Phys.* **1994**, *32*, 803.
(26) Flory, P. J.; Weaver, E. S. *J. Am. Chem. Soc.* **1960**, *82*, 4518 and references therein.
(27) Hara, M.; Takezoe, H.; Fukuda, A. *Jpn. J. Appl. Phys.* **1986**, *25*, 1756.
(28) Ohta, K.; Terazima, M.; Hirota, N. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2809 and ref 12 therein.
(29) Odulov, S. G.; Reznikov, Yu. A.; Soskin, M. S.; Khizhnyak, A. I. *Sov. Phys. JETP* **1983**, *58*, 1154.
(30) Kogelnik, H. *Bell Syst. Technol. J.* **1969**, *48*, 2909.


MA010436E