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Shear-induced color transition of PDA (polydiacetylene) liposome in polymeric solutions

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Abstract

The polydiacethylene (PDA) is known to change its color by mechanical shear. The shear-induced color transition has been reported with elastomer or film type of PDA. In this paper, we newly investigated the transition with liposome type of PDAs in polymeric solutions. The liposomes were dispersed in Poly(vinyl alcohol) 2% + Sodium borate 1%, Poly(vinyl alcohol) 15% and Hyaluronic acid 1% (PVA/B, PVA, HA). The shear stress was continuously imposed to each solution by stress control type rheometer with coni-cylinder fixture. The degree of color transition was quantified with the characteristic absorbance peak at 540 nm (blue) and 640 nm (red). As a result, PDA liposome in PVA/B solution changed the color from blue to red upon increasing the magnitude of shear (from 0 to 100 Pa) and the duration of shear-imposed time (from 0 to 5400 sec). Meanwhile, PDA liposome in HA or PVA solution did not noticeably change the color, even though the low shear viscosities of the solutions were kept almost constant. This color transition of PDA liposome is expected to measure the magnitude of shear, and to distinguish different responses of polymeric solutions to the applied shear.

Keywords : polydiacetylene (PDA), liposome, shear-induced color transition, normal stress difference

1. Introduction

The polydiacethylenes (PDAs) are fascinating materials to detect the perturbation from their surroundings. They exhibit visible color transition from blue to red in response to the perturbations such as temperature (Ahn *et al.*, 2003; Lio *et al.*, 1997; Rubner, 1986; Yuan *et al.*, 2006), binding of specific biological targets (Cheng and Stevens, 1997; Cheng and Stevens, 1998; Jelinek, 2000), pH (Kew and Hall, 2006) and molecule structure (Su *et al.*, 2004). The transition is known to originate from the alteration of PDAs' polymeric backbone conformation composed of alternating double and triple bonds. In optimal state, the conjugated polymer absorbs the light at nearly 640 nm, whose wavelength shows a blue color appearance. If the effective conjugation length is changed by perturbation, the maximum peak of absorption shifts to near 540 nm, which corresponds to bright red color appearance. This visible response is simple and fast so that the PDAs are used as a diagnostic sensor for chemical reaction, toxicity and so on.

Mechanical shear can invoke the color transition of PDAs (Carpick *et al.*, 2000; Nallicheri and Rubner, 1991; Tomioka *et al.*, 1989). Previously, PDAs were prepared as a type of elastomer or Langmuir-Blodgett film for investigation of shear-induced transition. Nallicheri and Rubner (1991) made use of poly(urethane-diacetylene) elastomer to observe the colorimetric difference between stretched state and relaxed state by the tensile strain. Tomioka *et al.* (1989) prepared PDA monolayer in Langmuir-Blodgett trough to investigate the reversible transition by surface pressure. Also, Carpick *et al.* (2000) used PDA molecular tri-layer films to observe the transition by force between AFM tip and PDA film.

Meanwhile, the liposome type PDA has little been paid attention to shear-induced color transition. The liposome is the structure composed of amphiphilic molecular bilayers that enclose a volume. The PDA liposome follows the same mechanism of color transition as we mentioned above, when perturbation is imposed on the liposomes: the color transition occurs from blue to red phase. It has the advantages of 1) being easily dispersed in target materials due to its amphiphilicity and 2) being highly sensitive because the perturbation easily transported to whole connected structures of liposome.

In this study, we attempt to observe the shear-induced color transition of PDA liposome. The liposomes were dispersed in three kinds of polymeric solutions: Poly(vinyl alcohol), Hyaluronic acid and PVA/Sodium borate. Also,

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we controlled the magnitude of shear and the duration of shear-exposure to associate with color transition.

2. Experimental section

2.1. Materials

We made the PDA liposome following Reichert *et al.* (1995). 10, 12 pentacosadiynoic acid was used as a diacetylene lipid. The liposomes were prepared using a probe sonication method and subsequently polymerized by irradiation at 254 nm. This PDA liposome is known to irreversibly change the color by perturbation (Ahn *et al.*, 2003). We prepared three polymer solutions, Poly(vinyl alcohol) (PVA, Sigma Aldrich, Mw: 124,000-186,000, 99 + % hydrolyzed), Hyaluronic acid (HA, presented from LG Life Sciences, Mw: 3,300,000) and PVA/Sodium borate (Borax, Sigma Aldrich, $Na_2B_4O_7$ $10H_2O$) dissolved in purified DI water. The zero shear viscosity of the solutions were kept constant at 40Pas, and the concentration was 15% of PVA, 1% of HA and 2%/1% of PVA/Sodium borate (PVA/B) in weight percentage, respectively. After dissolving the materials, PDA liposome were mixed and stirred softly at room temperature. The volume ratio of PDA liposome and each material solution was 1:20.

2.2. Instruments

The shear stress was controlled by a stress-control type rheometer (RS150, HAAKE) with coni-cylinder fixture (diameter = 20 mm). After imposing the shear stress continuously, we measured the absorbance at visible wavelength by the UV-VIS spectrometer (Spectronic® Genesys™ Spectrometers). Though we measured the absorbance after applying the shear in this experiment, the *in-situ* detection will be necessary to observe the process of color transition.

2.3. Shear-imposing

We imposed shear and measured colorimetric response in three steps. Firstly, the sample solution was put in the cup of coni-cylinder fixture at temperature 47°C. Secondly, the shear stress was continuously imposed in the duration of designated time. Finally, we measured the absorbance of shear-imposed sample.

2.4. Quantification of color transition

A quantitative measure of the extent of blue-red color transition is defined by the colorimetric response (CR), which was suggested by Charych *et al.* (1993),

$$CR = (PB_0 - PB_f)/PB_0 \times 100\%$$

where $PB = A_{blue}/(A_{blue} + A_{red}) \times 100\%$. If completely converted, CR value equals 100%. The symbol A is the absorbance at either the "blue" component in the UV-visible spectrum (640 nm) or the "red" component (540 nm) (Note: "blue" and "red" refer to the visual appearance of the material, not its relative absorbance). PB_0 is the PB of the control sample, while PB_f is the value obtained after external stimulus such as shear and temperature.

3. Results and discussion

The PDA liposome in PVA/B solution shows thermally induced color transition. Fig. 1(a) shows the spectral change as changing temperature from 40°C to 55°C. As the temperature increased, the intensity of the absorbance spectrum in the range of 620-640 nm (blue phase) becomes lower, while the intensity in the range of 530-550 nm (red phase) becomes higher. In other words, the color changes from blue to red. A shoulder peak appeared at around 580 nm in the range from 40°C to 47°C, but the peak diminished or shifted as increasing the temperature (50°C and 55°C). The phenomenon was quantified by colorimetric response (CR) in Fig. 1(b).

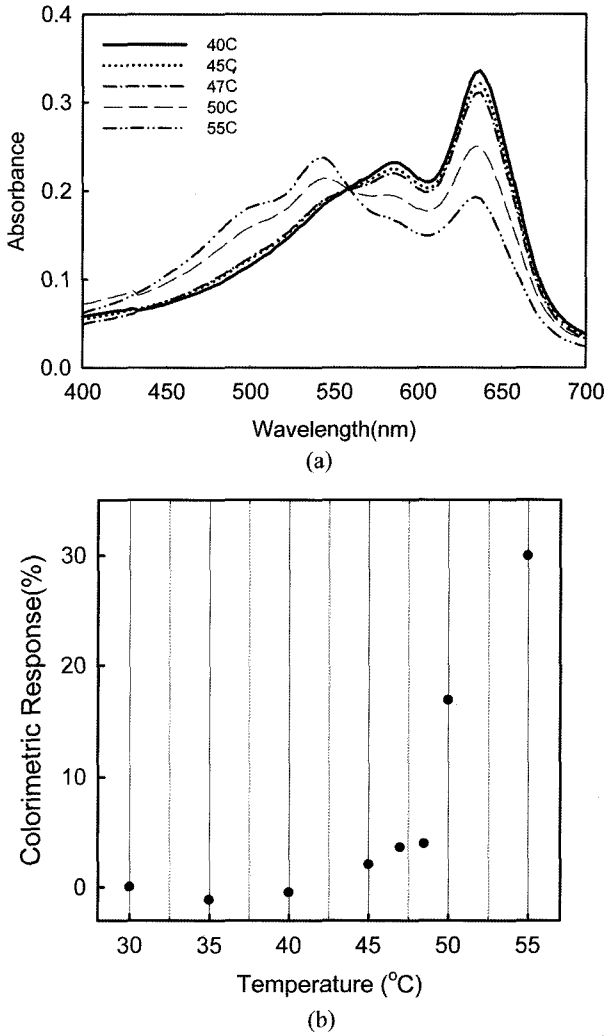
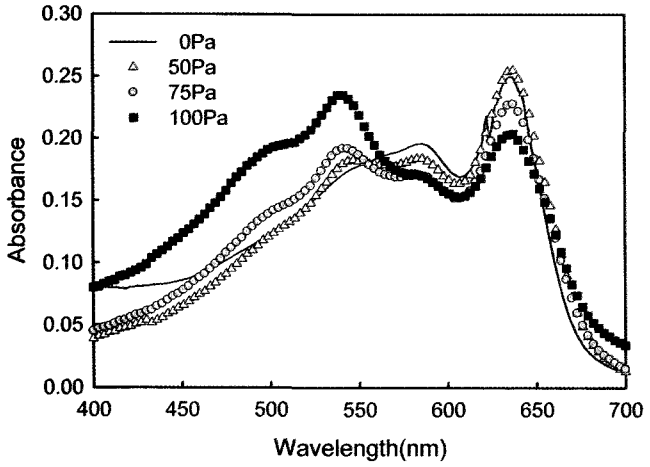


Fig. 1. Thermally induced color transition of PDA in PVA/B solution: (a) spectrum in the range of visible wavelength, (b) quantification by colorimetric response (CR) value.



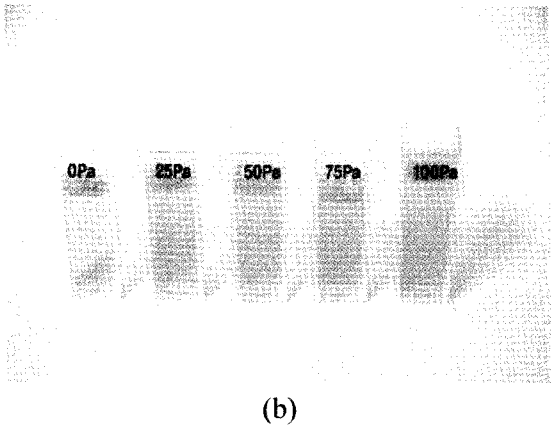


Fig. 2. Shear-induced color transition of PDA in PVA/B solution: (a) spectrum in the range of visible wavelength, (b) visible color transition.

We imposed shear to PVA/B solution. At low temperature (below 40°C), the shear did not contribute to color transition. Therefore, we imposed shear after making PDA liposome thermally excited (47°C). The condition seemed to be meta-stable state of PDA liposome before the abrupt color change which is known to appear at above 50°C. We expected that the shear could contribute to hurdle the transition barrier at meta-stable state.

To confirm our expectation, the shear-induced color transition was investigated for PVA/B solution at 47°C. We continuously imposed shear stress upon the solutions; increasing the magnitude from 0 to 100Pa. Fig. 2 clearly shows the effect of shear magnitude on the degree of transition. As the magnitude increased, a similar behavior was observed. The peak of the spectrum in the range of 620-640 nm (blue phase) became lower and the shoulder peak slightly moved to the red phase wavelength. The transition could be easily confirmed by the visual observation with naked eyes (Fig. 2(b)). The color of PDA in PVA/B solution turned purple as the response of large magnitude of

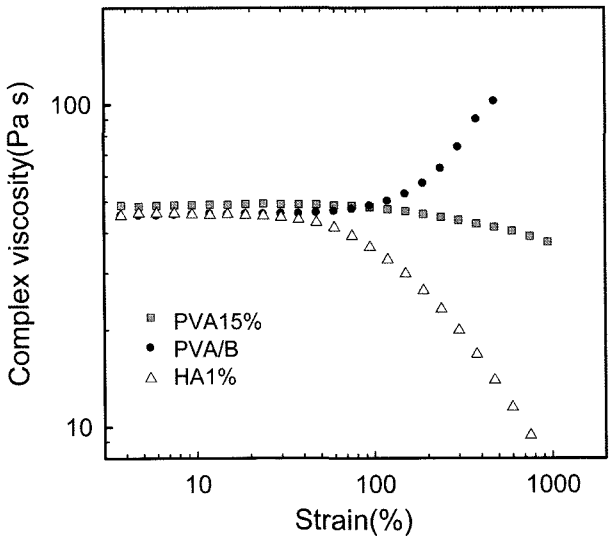


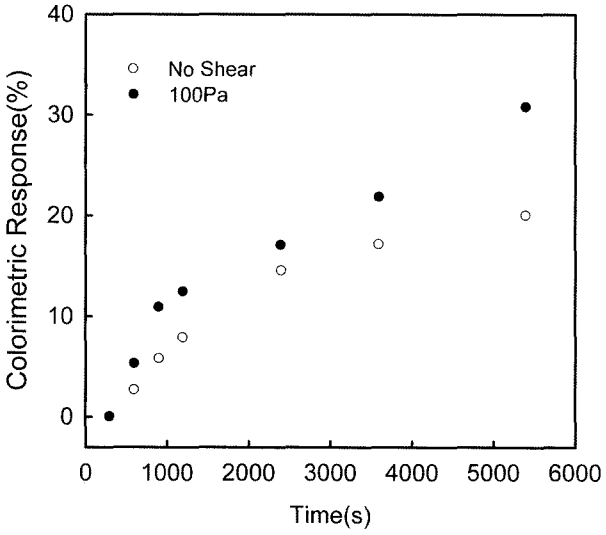
Fig. 3. Strain sweep results of the polymer solutions: PVA/B, PVA and HA solutions.

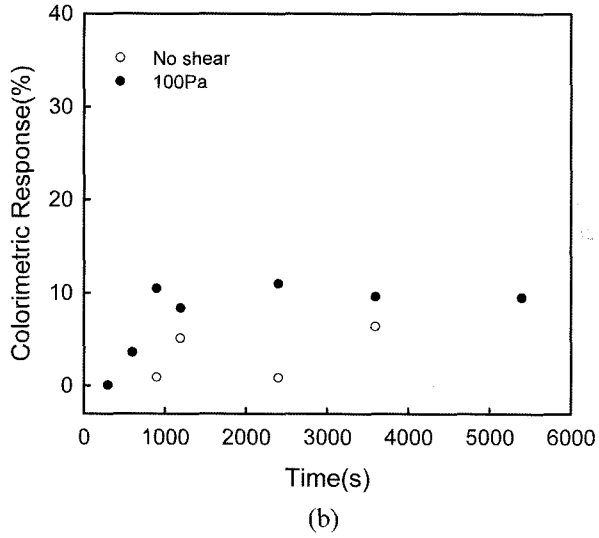
shear stress (75Pa, 100Pa). It is expected that the PDA liposome could be used as a sensor. The magnitude of imposed shear stress could be deduced from the value of colorimetric response (CR).

We expected the PDA could reflect the changes of the microstructure induced by shear stress and attempted to distinguish from different responses of polymeric solutions to the shear. For this purpose, three polymeric solutions (PVA/B, PVA and HA) were prepared. The viscosities of these materials were kept at the same level in low shear region (Fig. 3). Meanwhile, these materials responded differently in the large shear region. PVA/B, PVA and HA showed strong shear thickening, weak shear thinning and strong shear thinning under large magnitude of shear.

Large shear stress was imposed to each solution with PDA and the colorimetric responses were measured. (We could not impose 100Pa to HA solution due to mechanical limit of the instrument). Fig. 4 shows the colorimetric response of PDA in the solutions with and without shear stress as a function of time (the duration of shear-imposed time: 0-5400 sec). The contribution of shear stress was considered as the difference of CR between with and without shear. Comparing the shear contributions of the three solutions, PVA/B responded most sensitively to the shear. In case of PVA/B, the contribution of shear stress grows obviously as the imposed-time elapses. Meanwhile, the shear did not contribute to the color transition for PVA and HA solutions.

We presume this phenomenon to the specific response of PVA/B solution on the shear. PVA/B solution has been reported to form a network structure between ols and borate anions (Osaki *et al.*, 1994; Maeker and Sinton, 1986). The network strongly hinders from aligning of polymer chains against flow. Therefore, PVA/B shows strong





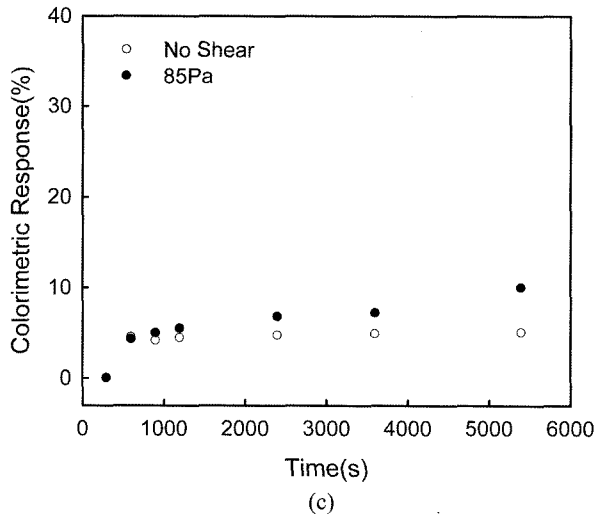


Fig. 4. Shear-induced colorimetric responses of PDA in polymer solutions: (a) PVA/B, (b) PVA, (c) HA.

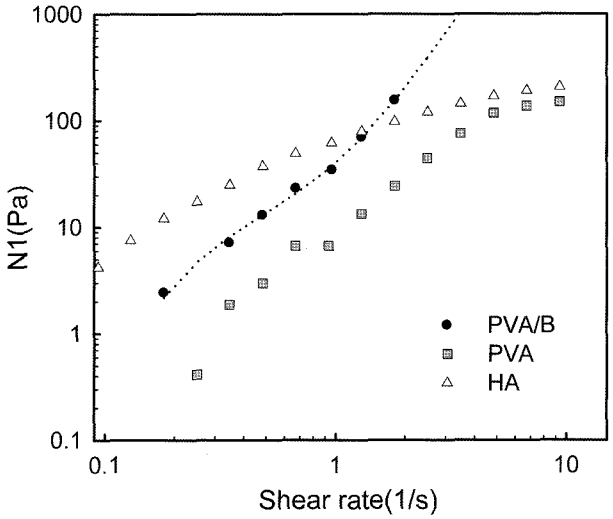


Fig. 5. First normal stress differences of PVA/B (dot line is only for visual help), PVA and HA solutions.

shear thickening phenomenon and the normal stress develops much higher than the other solutions in large shear region. Fig. 5 shows the first normal stress differences. Above shear rate of 2 s^{-1} (at this shear rate, the shear stress is about 100Pa), the first normal stress difference of PVA/B increases too abruptly to measure in the rheometer (overloaded). The large increase of stress of PVA/B solution at high shear is well known, and we added prediction at high shear with dotted line. In the simple shear flow, the stress contribution can be represented by either viscosity (1, 2-component of a stress tensor) or the first normal stress difference (difference between 1, 1 and 2, 2-component) and the second normal stress difference is trivial relative to the first normal stress difference. The detailed molecular mechanism is not yet clear, but the development of the normal stress will be closely related with the color transition of PDA solutions.

There are some limitations to be mentioned in this study. Firstly, we could not exactly separate the effect of thermal and mechanical stimuli on the color transition of PDA. The PDA liposome in this study was not sensitive enough to respond to mechanical shear (our experiment condition: below 100Pa) under thermally stable state. More sensitive PDA will be needed to fully understand the effect of mechanical shear on the color transition. Also, *In-situ* measurement will be helpful to separate the effect of thermal and mechanical perturbations. Another limitation is that our results underestimate the effect of shear on color transition. In the coni-cylinder fixture that was used in this study, the samples are subjected to non-uniform shear. Also the sample located at the bottom of the fixture experiences zero or lower magnitude of shear flow. Because both samples were mixed after removal from the inner cylinder, the effect of shear on color transition might be underestimated.

In spite of these limitations, this study is meaningful in that it firstly showed shear-induced color transition of PDA liposome. This phenomenon has a lot potential to visualize the shear stress distribution under flow and to design new devices. For example, it could contribute to design the artificial heart, in which the shear stress should be maintained below the threshold shear stress of blood damage. If PDA liposome would respond to shear stress above the threshold, it could be used as an indicator to evaluate the performance of the artificial heart.

4. Conclusions

In this study, we investigated the shear-induced color transition of PDA liposome dispersed in polymer solutions. The PDA liposome in PVA/B solution showed shear-induced color transition clearly as increasing the magnitude of shear and the duration of shear-imposed time. Meanwhile, PDA liposome in HA and PVA solutions did not noticeably change the color by shear, even though the viscosities of all the samples were kept constant at low shear. Though the shear-induced color transition was observed in thermally meta-stable state of PDA, this study is meaningful as a new attempt to apply PDA as a sensor for measuring the magnitude of shear and distinguishing the different rheological responses of polymer solutions to the applied shear.

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References

Ahn, D. J., E.-H. Chae, G. S. Lee, H.-Y. Shim, T.-E. Chang, K.-D. Ahn and J.-M. Kim, 2003, Colorimetric reversibility of polydiacetylene supramolecules having enhanced hydrogen-bonding under thermal and pH stimuli, *J. Am Chem. Soc.* **125**, 8976-8977.

Carpick, R.W., D.Y. Sasaki and A.R. Burns, 2000, First observation of mechanochromism at the nanometer scale, *Langmuir* **16**, 1270-1278.

Charych, D.H., J.O. Nagy, W. Spevak and M.D. Bednarski, 1993, Direct Colorimetric Detection of a Receptor-Ligand Interaction by a Polymerized Bilayer Assembly, *Science* **261**, 585-588.

Cheng, Q. and R.C. Stevens, 1997, Coupling of an induced fit enzyme to polydiacetylene thin films: Colorimetric detection of glucose, *Adv. Mater* **9**, 481-483.

Cheng, Q. and R.C. Stevens, 1998, Charge-induced chromatic transition of amino acid-derivatized polydiacetylene liposomes, *Langmuir* **14**, 1974-1976.

Jelinek, R., 2000, Colorimetric sensors for drug discovery and biomedical diagnostics, *Drug. Develop. Res.* **50**, 497-501.

Kew, S.J. and E.A.H. Hall, 2006, pH response of carboxy-terminated colorimetric polydiacetylene vesicles, *Anal. Chem.* **78**, 2231-2238.

Lio, A., A. Reichert, D.J. Ahn, J.O. Nagy, M. Salmeron and D.H. Charych, 1997, Molecular imaging of thermochromic carbohydrate-modified polydiacetylene thin films, *Langmuir* **13**, 6524-6532.

Maerker, J.M. and S.W. Sinton, 1986, Rheology Resulting from Shear-Induced Structure in Associating Polymer-Solutions, *J. Rheol.* **30**, 77-99.

Nallicheri, R.A. and M.F. Rubner, 1991, Investigations of the Mechanochromic Behavior of Poly(Urethane Diacetylene) Segmented Copolymers, *Macromolecules* **24**, 517-525.

Osaki, K., T. Inoue and K.H. Ahn, 1994, Shear and Normal Stresses of a Poly(Vinyl Alcohol) Sodium Borate Aqueous-Solution at the Start of Shear-Flow, *J. Non-Newton Fluid* **54**, 109-120.

Reichert, A., J.O. Nagy, W. Spevak and D. Charych, 1995, Polydiacetylene Liposomes Functionalized with Sialic-Acid Bind and Colorimetrically Detect Influenza-Virus, *J. Am Chem. Soc.* **117**, 829-830.

Rubner, M.F., 1986, Novel Optical-Properties of Polyurethane Diacetylene Segmented Copolymers, *Macromolecules* **19**, 2129-2138.

Su, Y.L., J.R. Li and L. Jiang, 2004, Effect of amphiphilic molecules upon chromatic transitions of polydiacetylene vesicles in aqueous solutions, *Colloid Surface B* **39**, 113-118.

Tomioka, Y., N. Tanaka and S. Imazeki, 1989, Surface-Pressure-Induced Reversible Color-Change of a Polydiacetylene Monolayer at a Gas Water Interface, *J. Chem. Phys.* **91**, 5694-5700.

Yuan, Z.Z., C.W. Lee and S.H. Lee, 2006, Reversible thermochromism in self-layered hydrogen-bonded polydiacetylene assembly, *Polymer* **47**, 2970-2975.