

Self-Oscillating Soluble–Insoluble Changes of a Polymer Chain Including an Oxidizing Agent Induced by the Belousov–Zhabotinsky Reaction

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A new type of self-oscillating polymer was prepared by utilizing the Belousov–Zhabotinsky reaction. In this study, capture sites with a positive charge for an oxidizing agent as a counterion were incorporated into the copolymer of *N*-isopropylacrylamide and the ruthenium complex as a catalyst. Soluble–insoluble self-oscillation of the polymer was first achieved without adding an oxidizing agent. The effect of temperature on the self-oscillating behavior was investigated. It was clarified that the polymer had two advantageous characteristics because of the higher LCST; one is to enable self-oscillation around body temperature, and the other is to cause the oscillation for a longer time without intermolecular aggregation among the polymer chains in the reduced state. This achievement of self-oscillation of polymer chains including an oxidizing agent may lead to their practical use under oxidant-free conditions.

Introduction

In a living body, the mechanisms generating autonomous and stimuli-responsive activities are inherent. From the standpoint of biomimetic materials design, functional polymer systems have been extensively studied¹ especially for the purpose of application to biomedical fields such as drug delivery systems,^{2,3} biosensors,^{4,5} and actuators (artificial muscle),^{6–8} etc. However, although many studies on stimuli-responsive polymer systems are reported, there are few studies on autonomous systems which exhibit rhythmic changes under constant condition. If such materials can be created, novel bioinspired materials would be realized.

To develop intelligent material systems with autonomy, we focused on the Belousov–Zhabotinsky (BZ) reaction, which is well-known as an oscillating chemical reaction to generate rhythm and pattern^{9–11} and often analogically compared with the TCA cycle (Krebs cycle) which is a key metabolic process taking place in the living body. The overall process is the oxidation of an organic substrate by an oxidizing agent in the presence of the catalyst under acidic conditions. In the reaction process, a cyclic reaction network of intermediates is created and the catalyst undergoes spontaneous redox oscillations.

For the design of self-oscillating polymers and gels by utilizing the BZ reaction, ruthenium tris(2,2'-bipyridine) (Ru(bpy)₃) was incorporated as the catalyst into poly(*N*-isopropylacrylamide) (PNIPAAm). With the redox oscillation of the catalyst moiety, the hydrophilicity of the polymer chain changes periodically. As a result, autonomous and spontaneous swelling–deswelling^{12–17} or soluble–insoluble oscillations^{18–21} were achieved under constant condition with coexistence of the substrates for the poly(NIPAAm-*co*-Ru(bpy)₃) gel and the linear polymer, respectively.

At the present stage, an operating condition for the self-oscillation is limited to the nonphysiological environment where

the three substrates of the BZ reaction (malonic or citric acid, sodium bromate as an oxidant, and sulfuric or nitric acid) coexist. If the self-oscillation can be caused under physiological condition only in the presence of biorelated organic acids such as citric acid, unprecedented applications as novel intelligent biomaterials would be expected.

To cause the self-oscillation of the polymer under physiological conditions without utilizing nonbiorelated BZ substrates, we have been attempting to construct the build-in system where the substrates other than organic acid are incorporated into the polymer itself. One method is to introduce a pH-control site into the polymer. In the previous study,^{20,21} acrylamide-2-methylpropanesulfonic acid (AMPS) was incorporated into the poly(NIPAAm-*co*-Ru(bpy)₃) as the pH-control site. Autonomous soluble–insoluble self-oscillation of the polymer was achieved under acid-free conditions in which only the two BZ substrates (i.e., malonic acid and sodium bromate) were present as added agents. It was demonstrated that the oscillating behavior for the AMPS-containing polymer is controllable by changing the polymer concentration, the polymer composition, and temperature.

In this study, as the next step for the final purpose, we have attempted to introduce the oxidizing agent into the polymer. (Methacrylamido)propyltrimethylammonium chloride (MAPTAC) with a positively charged group was incorporated into the poly(NIPAAm-*co*-Ru(bpy)₃) as a capture site for an anionic oxidizing agent (bromate ion). The bromate ion was introduced into the MAPTAC-containing polymer through ion-exchange process. Under the conditions in which only the two BZ substrates (i.e., malonic acid and sulfuric acid) were present, soluble–insoluble self-oscillation of the polymer was measured. The effect of temperature on the self-oscillating behavior was investigated.

Experimental Section

Polymerization. Using NIPAAm (2.5 g), MAPTAC (6.5 g), Ru(bpy)₃ monomer (ruthenium (4-vinyl-4'-methyl-2,2'-bipyri-

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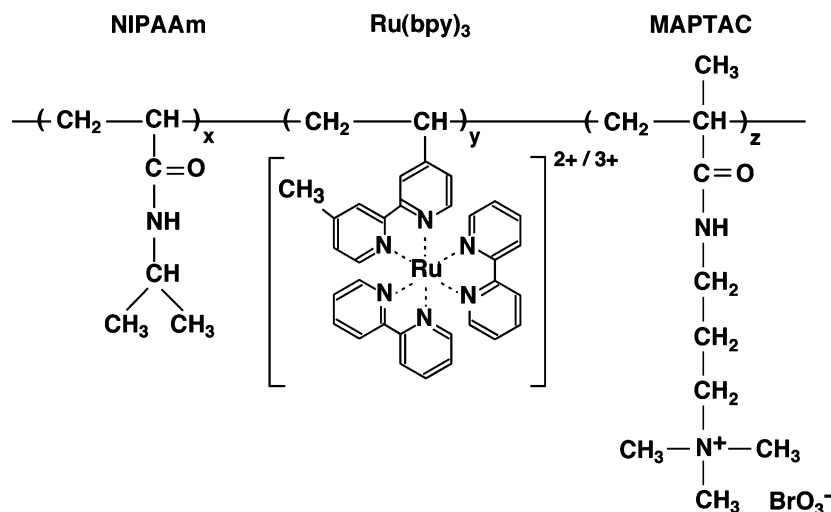


Figure 1. Chemical structure of poly(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC).

dine)bis(2,2'-bipyridine)bis(hexafluorophosphate)) (1.0 g), and 2,2'-azobis(isobutyronitrile) (AIBN) (0.35 g) as an initiator, poly(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC) (Figure 1) was synthesized by radical polymerization in methanol (40 g). The polymerization was carried out at 60 °C for 24 h in vacuo. The resulting reaction mixture was dialyzed against methanol for 3 days and then water for 4 days. For exchanging the counterion, the polymer was dissolved in NaBrO₃ aqueous solution (1 M) and dialyzed against pure water for 30 days with repeating exchanging the water to remove excess Na⁺ and BrO₃⁻ ions and then freeze-dried.

Measurements of LCST. The lower critical solution temperature (LCST) of the polymer solution was measured under reduced and oxidized states, by using Ce(SO₄)₂ as an oxidizing agent and Ce₂(SO₄)₃ as a reducing agent, respectively. Polymer solutions (5.0 wt %) were prepared by dissolving the polymer in an aqueous solution containing 2.0 mM Ce(SO₄)₂ or 2.0 mM Ce₂(SO₄)₃, respectively. LCST measurements were carried out with a spectrophotometer (Shimadzu, model UV-2500) equipped with a thermostatic controller and magnetic stirrers. To detect the optical transmittance change which is based on the soluble–insoluble change, the 570 nm wavelength was used, because it is the isosbestic point for the reduced and oxidized states of the Ru(bpy)₃. The transmittance (%) of the polymer solution at 570 nm was then recorded by raising the temperature at a rate of 0.5 °C/min.

Measurements of Optical Oscillations. Polymer solutions were prepared by dissolving the polymer (5.0 wt %) into an aqueous solution containing the two BZ substrates (0.1 M malonic acid and 0.3 M sulfuric acid). The optical transmittance oscillations for the polymer solutions were measured under constant temperature and stirring. The time course of transmittance at 570 nm was monitored by a spectrophotometer.

Results and Discussion

The aqueous solution of PNIPAAm exhibits LCST around 31 °C.²² In the previous study, it was shown that the LCSTs for the poly(NIPAAm-*co*-Ru(bpy)₃) in the oxidized and reduced states shifted higher and lower than the LCST of PNIPAAm, respectively.¹⁸ The rise in the LCST by oxidation is due to an increase in hydrophilicity of the polymer by the charge increase of the catalyst. This phenomenon is generally observed in NIPAAm-based polyelectrolytes when their ionic charges increase. In the reduced state, however, since the effect of the hydrophobicity of the bipyridine ligand surrounding the ruthenium

ion becomes dominant, the polymer becomes more hydrophobic than PNIPAAm and consequently the LCST decreases. Namely, the redox states of the Ru(bpy)₃ significantly affect the solubility of the polymer chains.

When the positively charged MAPTAC is introduced into the poly(NIPAAm-*co*-Ru(bpy)₃), the hydrophilicity of polymer chain increases as a whole because the ratio of cationic site increases. As a result of LCST measurements, the LCST for the poly(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC) in the reduced state was 45 °C, which is much higher than that for the poly(NIPAAm-*co*-Ru(bpy)₃). On the other hand, in the oxidized state, the LCST disappeared because the polymer becomes highly hydrophilic.

Figure 2 shows the transmittance change for the solutions of MAPTAC-containing polymer at several temperatures from 15 to 45 °C under coexistence of only the two BZ substrates (malonic acid and sulfuric acid). Self-oscillation of optical transmittance due to soluble–insoluble changes of the polymer on the basis of different solubilities between reduced and oxidized states was observed. Since the polymer supplies the oxidizing agent (BrO₃⁻) by itself as a counterion to MAPTAC site, self-oscillation was achieved without adding oxidizing agent.

Since the poly(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC) has the LCST around 45 °C in the reduced state, the polymer has an advantage to enable self-oscillation around body temperature because aggregation of the polymer does not occur at the reduced state. This characteristic is significantly important for applications to intelligent biomaterials. Further, it is a special tendency for the MAPTAC-containing polymer that the self-oscillation continues for a longer time compared to the other self-oscillating polymers we have studied previously such as poly(NIPAAm-*co*-Ru(bpy)₃) and poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS).^{18–21} For example, the oscillation lasted more than 3 h at 27 °C (Figure 2c). The longer duration of self-oscillation is also attributed to higher LCST of the polymer to avoid aggregation due to intermolecular hydrophobic interaction at reduced state. Differently from poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS), the polymer has no anionic site, which also contributes to prevent aggregation due to the formation of polyion complex. As the temperature approaches to 45 °C (the LCST in reduced state), damping of the oscillation becomes remarkable as shown in Figure 2. Around the LCST, the polymer chains easily aggregate in the reduced state. Once the polymer aggregates, they could not be easily elongated even in the oxidized state

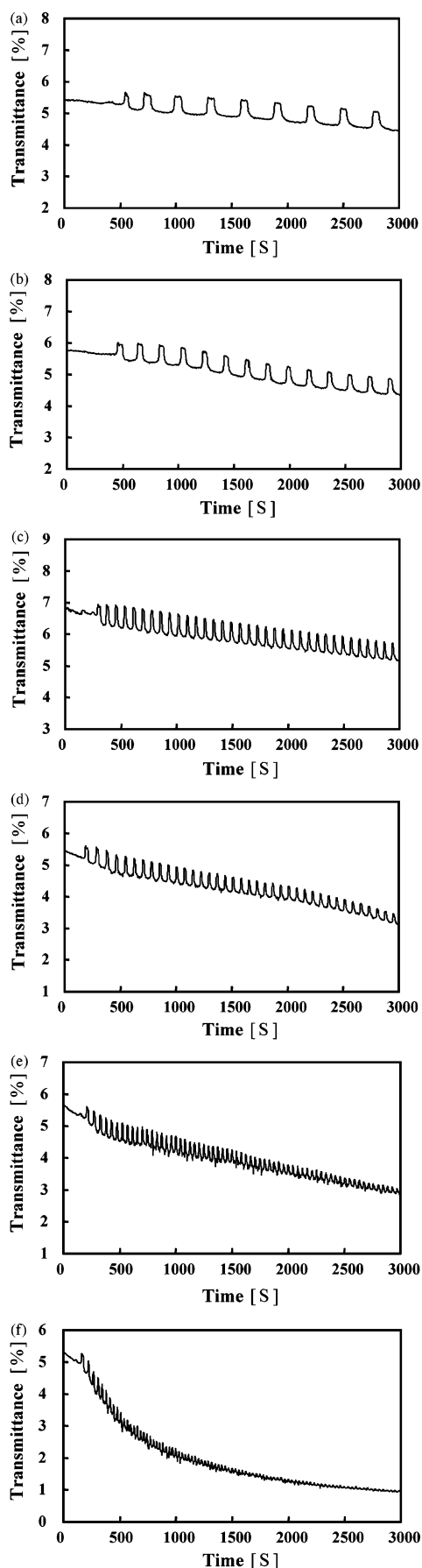


Figure 2. Oscillating profiles of the optical transmittance for poly-(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC) solutions at several constant temperatures: (a) 15 °C; (b) 21 °C; (c) 27 °C; (d) 33 °C; (e) 39 °C; (f) 45 °C.

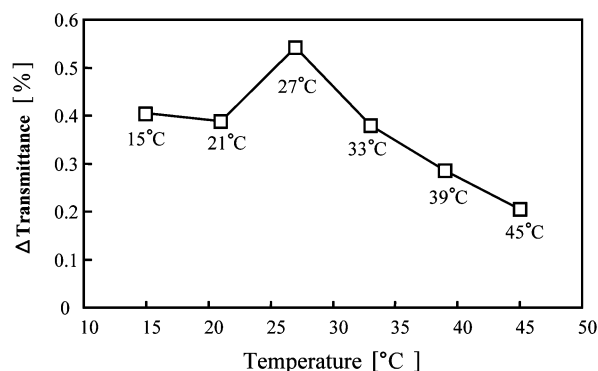


Figure 3. Dependence of the oscillation amplitude ($\Delta T\%$) on temperature.

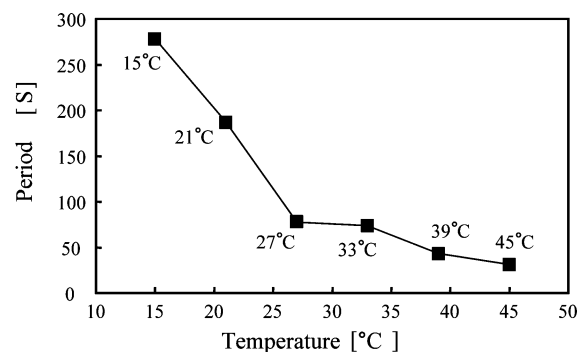


Figure 4. Dependence of the oscillation period on temperature.

because of strong hydrophobic interaction. The reduction of reversibility for conformational changes leads to the damping of self-oscillation.

The amplitudes ($\Delta T\%$) of the self-oscillation for several temperatures were summarized in Figure 3. All over the temperature range, the amplitude for MAPTAC-containing polymer was much smaller than that for the other self-oscillating polymers we have studied previously.^{18–21} The driving force of the soluble–insoluble self-oscillation is the difference in solubility of the polymer between oxidized and reduced states. In the case of MAPTAC-containing polymer, the solubility in the reduced state increases due to higher LCST. Consequently the solubility difference becomes smaller, which may be main reason for small amplitude.

The oscillation periods were plotted as a function of temperature (Figure 4). The period decreased with increasing temperature. This is a general tendency for the BZ reaction as a well-known phenomena that the period typically follows the Arrhenius equation.^{23–25} Aside from the temperature dependence, it is characteristic for MAPTAC-containing polymer that the period is shorter compared to the other self-oscillating polymers. Especially, a period with several tens of second has not been observed in the previous studies. The smaller amplitude and shorter period observed for the poly(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC) systems may be attributed that the oscillation occurs at relatively lower temperature region compared to the LCST in the reduced state (45 °C). At these temperatures, the polymer chains still have high mobility even in the reduced state enough to cause quick conformational changes. From these results of Figures 3 and 4, it is suggested that the self-oscillating behavior of the MAPTAC-containing polymer is controllable by adjusting temperature.

Our next interest is whether it is possible to incorporate both of the pH-control and the oxidant-supplying sites into one polymer chain at the same time. For this purpose, we are now

preparing a novel polymer, i.e., poly(NIPAAm-co-Ru(bpy)₃-co-AMPS-co-MAPTAC), and investigating the physicochemical properties. Since the polymer has both of anionic and cationic sites, the formation of polymer complex to cause precipitation is predicted. But so far it has been confirmed that complex formation does not occur because the net charge balance is different. The details will be discussed in the next report. If this polymer can be utilized, self-oscillation only in the presence of biorelated organic acid might be possible. As a step toward the final goal, in this study, self-oscillation utilizing poly(NIPAAm-co-Ru(bpy)₃-co-MAPTAC) was demonstrated.

In the polymer solution system, the oxidant (bromate ion) as a counterion can diffuse throughout the solution. As a material systems for practical use, it would be better to trap the ions in the polymer network by cross-linking the polymer, which also meets our original purpose to construct chemomechanical systems. In the case of gel, the oxidant exists as a mobile counterion in the polymer network. Although we must still consider a method to prevent the diffusing out of the ions from the gel, the introduction of MAPTAC would be available to capture the oxidizing agent into the polymer in advance.

Conclusions

In the self-oscillating polymer solution system induced by the BZ reaction, self-oscillation was achieved without adding the oxidizing agent by utilizing the MAPTAC-containing polymer which includes sodium bromate as a counterion. The self-oscillating behavior is controllable by temperature. The polymer has two advantageous characteristics which derive from their higher LCST; one is to enable self-oscillation around body temperature, and the other is to cause the oscillation for a longer time without intermolecular aggregation among the polymer chains in the reduced state. A step toward the practical use of self-oscillating polymers under physiological conditions as novel smart materials has been established.

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