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Novel Hydrogels for Rhythmic Pulsatile Drug Delivery

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Summary: We are investigating an autonomous glucose-driven hydrogel/enzyme-based device prototype for rhythmic, pulsed delivery of gonadotropin releasing hormone (GnRH). The device employs a pH-sensitive hydrogel membrane in conjunction with the enzyme glucose oxidase. This system delivers GnRH in rhythmic pulses when exposed to a constant level of glucose. These pulses result from autonomous pH oscillations inside the device that are created by an unstable nonlinear feedback between hydrogel permeability to glucose and production of acid by glucose oxidase. Previous versions of this prototype utilized p(*N*-isopropylacrylamide-*co*-methylacrylic acid) p(NIPA-*co*-MAA) hydrogels, with 10 mol% MAA incorporated. With this membrane, which undergoes a volume transition (VT) near pH 5, pH oscillations centered around pH 5 are observed. This range is too low to sustain oscillations in physiologically buffered media. To shift the operating pH of oscillations closer to physiologic pH, we have sought ways to increase the pH of the volume transition. In this study we show that increasing the side chain length of the α-alkylacrylic acid (RAA) comonomer enhances the overall hydrophobicity of the copolymer, and shifts the VT pH closer to physiological pH values. We also demonstrate the ability of such membranes to affect an alkaline shift in the range of oscillations in the prototype oscillator device.

Keywords: hydrogels; hydrophobic; pH-sensitive; *N*-isopropylacrylamide; pulsatile

Introduction

Poly(*N*-isopropylacrylamide) [p(NIPA)] hydrogels exhibit a sharp volume transition (VT) in response to infinitesimal changes in temperature.[1–4] NIPA can be copolymerized with other comonomers to produce gels that can undergo such volume transitions in response to other external stimuli such as pH, electric field and light.[5–7] Hydrogels based on these stimuli-sensitive materials have been investigated for controlled gene,[8] peptide[9] and drug delivery,[10] protein purification,[11] sensors,[12] and chromatography.[13]

We have been developing an autonomous, glucose-powered hydrogel-based device for rhythmic, pulsed release of gonadotropin releasing hormone (GnRH) that utilizes pH-sensitive copolymers of N-isopropylacrylamide and carboxylic acids.[14–17] For example, copolymer hydrogels consisting of poly(*N*-isopropylacrylamide-*co*-methacrylic acid) [poly(NIPA-*co*-MAA)] with 10 mol.% MAA exhibit a volume transition (VT) at 37 °C around pH 5.0.[15] When these hydrogels are cast as membranes, they exhibit sharp changes in permeability to glucose near pH 5.0.[14–18] Placing such a membrane into a side-by-side diffusion cell (see Figure 1), with the donor side fed continuously with constant concentration glucose solution at pH 7.4, and with the receptor cell containing glucose oxidase and solid marble ($CaCO_3$), pH oscillations in the receptor cell are generated.[16,19] Further, when GnRH is incorporated into the receptor cell, the hormone is released in rhythmic pulses that are coherent with the pH oscillations, indicating

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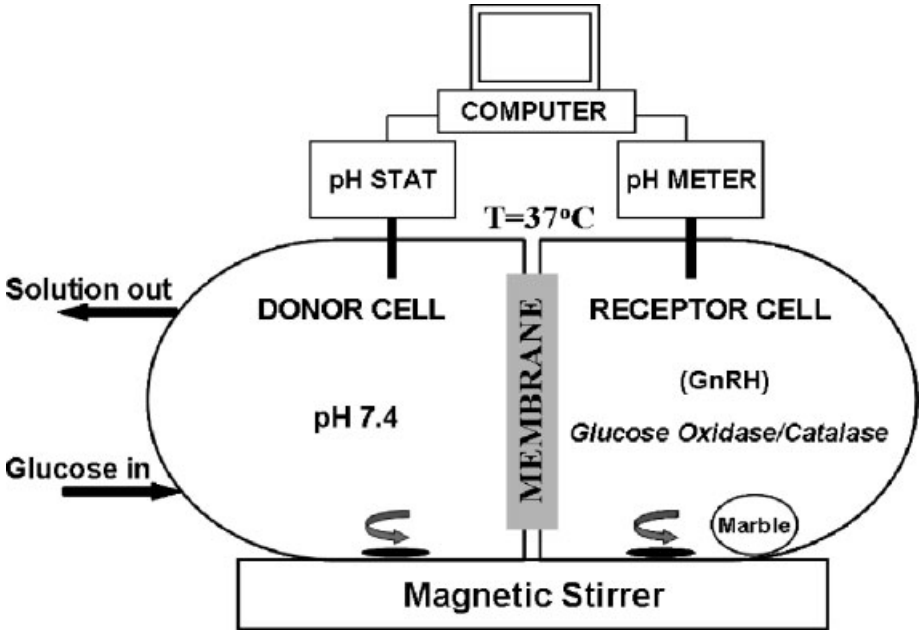

Figure 1.
Schematic of glucose-driven chemomechanical oscillator. "GnRH" is in parentheses to indicate its absence in present experiments.

that the hydrogel is repetitively swelling and shrinking.[16,19]

The mechanism of oscillation is as follows. When pH in the receptor cell is above the VT pH, the hydrogel is charged and swollen, and glucose rapidly crosses the membrane and reacts with glucose oxidase to form gluconic acid. Subsequent dissociation produces gluconate$^-$ and H^+, which lowers pH in the receptor cell. As H^+ diffuses into the hydrogel, it protonates pendant carboxylate groups (MAA) and reduces the charge on the membrane. Eventually the hydrophobic NIPA component dominates, and the membrane collapses, blocking further glucose influx. The remaining H^+ in the receptor cell then either diffuses out or reacts heterogeneously with the marble, and pH rises.[15] Meanwhile, the carboxylic acid groups in the hydrogel dissociate and H^+ diffuses into the donor cell. As the hydrogel recharges, it reswells to its initial state, and the system has completed one period of oscillation. It is now poised to repeat the same sequence of events over and over.

Currently, buildup of gluconate$^-$ in the receptor cell over time limits the long-term operability of the prototype device,[20] due to buffering of pH swings, which leads to slowing and ultimate freezing of pH oscillations. Gluconate$^-$ buildup is exacerbated by the relatively high glucose concentration (50 mM) that is currently employed in operating the device. One way to overcome this problem is to shift the VT pH, and hence the range of the pH oscillations in the receptor cell closer to physiological pH values. Since less glucose is required to produce the higher pH values in the receptor cell, less gluconate$^-$ will be generated, and the buffer problem will be reduced substantially.

Swelling behavior of pH-sensitive hydrogels is dictated by a balance of forces involving elasticity of the polymer network, polymer-solvent mixing, and ionic osmotic pressure,[21] the latter increasing with charge on the hydrogel network. With this balance in mind, it follows that increased network charge must be compensated by increased hydrophobicity in order to

produce equivalent swelling degree. Furthermore, network charge is determined by the degree to which ionizable groups are incorporated, the dissociation constant (pKa) of the ionizable groups, and pH. In particular, for hydrogels with acidic sidechains, ionization increases either with increasing sidechain substitution or with increased value of pH-pKa. From these heuristics, we hypothesize that VT pH will increase either with increasing hydrophobicity of the polymer network, or with decrease in acidic sidechain content.

Based on this hypothesis, we have synthesized copolymer hydrogel membranes of NIPA with higher order homologs of MAA and compared their pH-dependent equilibrium swelling behaviors at 25 °C and 37 °C. Substituting the α-methyl group in MAA by alkyl groups of increasing length, hydrophobicity of the hydrogel increased. Hydrophobicity is decreased by lowering temperature. We also varied degree of substitution of the acidic comonomer in order to test the proposed effect on VT pH. Finally, the range of pH oscillations in the device prototype was correlated with VT pH.

Experimental Part

Materials

N-isopropylacrylamide (NIPA) (Polysciences, Inc.) was recrystallized from a heptane/acetone mixture.[11] Ammonium persulfate (APS) and ethyleneglycol dimethacrylate (EGDMA; Polysciences) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED; Sigma Co.) were used as received. Acrylic acid (AA; Aldrich) and methacrylic acid (MAA; Aldrich) were vacuum distilled to remove *p*-methoxyphenol. Glucose oxidase (90 IU/mg) and catalase (13610 IU/mg) were obtained from Sigma. All buffers were prepared with deionized water (Millipore Milli-U-10, >18MΩ resistivity).

Monomer Synthesis

Ethacrylic acid (EAA), *n*-propylacrylic acid (PAA) and *n*-butylacrylic acid (BAA) were synthesized by the Mannich reaction of the corresponding diethylakylmalonates (Aldrich) and subsequent hydrolysis, using procedures adapted from Ferrito and Tirrell.[22] Following distillation under reduced pressure, structures of the newly synthesized monomers were confirmed by ^{1}H-NMR.

Copolymerization

Copolymer hydrogels were synthesized by redox free radical polymerization. Hydrogels are designated Rxx where R is the first letter of the acronym for the acidic comonomer (R = A, M, E, P, or B) and xx corresponds to the mol% RAA in the feed composition. Pregel solutions were formed by dissolving a total of 3.92 mmol of NIPA and RAA, with respective contents determined by xx, along with 5.26 μL EGDMA (crosslinker), all in an 880 μL methanol/water mixture. The complete pre-gel solution was purged with nitrogen gas and sonicated for 3 minutes. Following addition of 4.4 mg of APS (initiator), and 17.55 μL TEMED (accelerator), the pre-gel solution was immediately transferred between two Teflon-spaced (400 μm) silanized glass plates, and polymerization was carried out overnight at 4 °C (in a refrigerator) or at 25 °C (in the laboratory). See Table 1 for precise polymerization conditions (solvent composition and temperature) for each hydrogel.

Table 1.
Composition of the NIPA/RAA hydrogels based on elemental analysis results. Polymerization condition includes temperature and solvent composition (vol/vol MeOH/H_2O) during synthesis.

Hydrogel	Polymerization condition	Polymer acid mol%
A05	4 °C, 50/50	6.0
M05	4 °C, 50/50	12.5
E05	4 °C, 70/30	14.9
P05	25 °C, 70/30	3.4
B05	25 °C, 70/30	13.6
A10	4 °C, 50/50	17.4
M10	4 °C, 50/50	23.3
E10	4 °C, 70/30	32.5
P10	25 °C, 70/30	29.8
B10	25 °C, 70/30	29.6

The resulting hydrogel slabs were carefully removed and washed in a series of methanol/water baths to remove unreacted chemicals. Copolymer contents, determined by elemental analyses (MHW Labs, AZ), are shown in Table 1.

Swelling Characterization

Cylindrical disks (11 mm diameter) were cut from the slabs, and were equilibrated in 155 mM buffer solutions at 37 °C and specified pH values. Buffers consisted of 10 mM acetate (pH 4–5.5), MES (pH 5.5–6.2), or phosphate (6.2–7.6), and were balanced to 155 mM ionic strength with NaCl. After four days, disks were carefully removed and weighed, after tapping off excess water. The hydrogels were then dried in a vacuum oven at 50 °C for a week to determine dry weights. Swelling ratios of the hydrogels were calculated by dividing wet weights by dry weights. Room temperature swelling studies for 5% PAA and 5% BAA hydrogels were carried out in a similar manner.

pH Oscillations

The glucose powered chemomechanical oscillator setup consisted of a clamped side-by-side diffusion cell (Crown Glass) (100 mL each) with the pH-sensitive hydrogel membrane (400 μm thickness when synthesized) mounted between the donor and receptor cells, as illustrated in Figure 1. Saline (50 mM) containing a fixed concentration of glucose was flowed through the donor cell at 1.4 mL/min, and pH in the donor cell was fixed at 7.4 using a pH-stat. The receptor cell contained 20 mg glucose oxidase and 2 mg catalase (also containing trace amounts of gluconolactonase) along with a piece of marble ($CaCO_3$), all in 80 ml saline. The exposed membrane area between the two diffusion cells was 3.14 cm^2. The entire set-up was maintained at 37 °C and both cells were magnetically stirred (600 rpm). Changes in the pH in the receptor cell were monitored by a pH-electrode and digitally recorded. GnRH was not included in the present experiments.

Results and Discussion

Swelling Studies

Equilibrium swelling curves at 37 °C for copolymers hydrogels with 5% and 10% RAA are shown in Figure 2a and 2b, respectively. All hydrogels underwent a sharp volume transition at characteristic pH values (VT pH). A stepwise alkaline shift in VT pH of about 0.5 pH units/α-methylene group on the acidic comonomer was observed, for a given degree of substitution. Comparing hydrogels with the same α-alkylacrylic acid but different degrees of substitution (corresponding curves in Figure 2a,b), alkaline shifts in VT pH by 0.2–0.5 units are observed.

The results are in line with our initial hypothesis, since reduced RAA content leads to fewer ionizable groups, and longer alkyl side chains engender greater hydrophobicity of the network. Based on the extremely weak dependence of pKa of alkanoic acids on alkyl chain length,[23] the observed pH-shifts in swelling response should not be attributed to changes in intrinsic pKa.

At low pH values where hydrophobic forces dominate, the hydrogel is collapsed. With increasing pH, acidic groups fixed on the polymer chains ionize, increasing the ionic osmotic swelling force. When a critical fixed charge density is reached at the VT pH, the osmotic swelling force overcomes the net hydrophobic collapsing force, and the hydrogel undergoes a transition to the swollen state. Copolymerizing NIPA with higher homologs of acrylic acid (methyl-, ethyl-, propyl- and butylacrylic acids) increases the hydrophobic force, and an increased fixed charge density is needed for the hydrogel to swell. This requirement can be realized either by increasing the availability of ionizable groups (increased substitution of RAA), or by increasing the proportion of acid groups that are ionized (increased pH). The forgoing argument explains the alkaline shift in the VT pH of the gels that occurs with increasing hydrophobicity and/or decreasing substitution of acidic groups in the hydrogel.

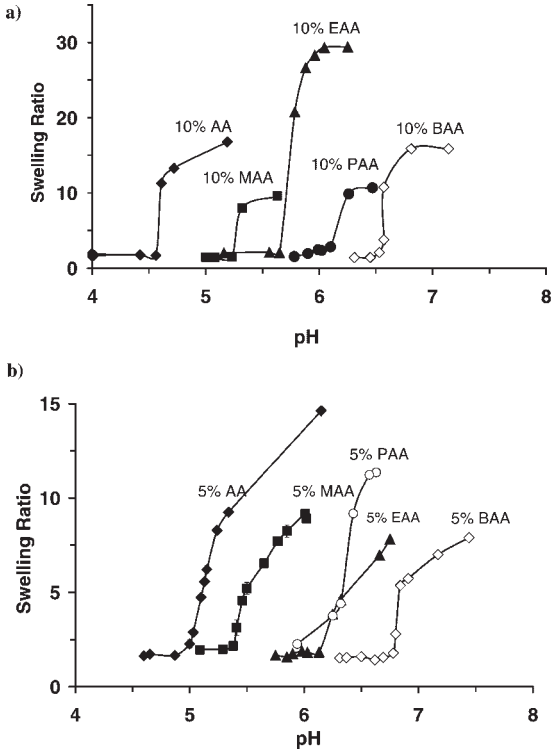

Figure 2.
pH-dependent equilibrium swelling studies on poly(NIPA-*co*-RAA) hydrogels at 37 °C. a) 5 mol% RAA (R05) copolymer membranes; b) 10 mol% RAA (R10) copolymer membranes.

The effect of network composition on ultimate swelling ratio above VT pH is not well defined by the data in Figure 2a,b. Based on hydrophobicity alone, one might expect that with the ultimate swelling ratio would increase with hydrophobicity of the RAA comonomer,[15,21] but no such trend was observed. Within the R10 series, the E10 hydrogels, which are of intermediate hydrophobicity, exhibited the highest swelling above VT pH. Elemental analyses (Table 1) revealed that the copolymer composition differed from the corresponding feed composition, in a manner varying from gel to gel. The exceptionally high swelling of the E10 hydrogel can thus be attributed to enrichment (32% mol) of EAA into the hydrogel compared to its feed composition (10 mol%). A likely cause for discrepancies in RAA incorporation across hydrogels is variation in polymerization conditions such as temperature and solvent composition.

A comparison of pH-dependent swelling equilibria for P05 and B05 hydrogels at 25 °C and 37 °C is presented in Figure 3. Three salient trends are observed. First, increasing temperature is accompanied by an alkaline shift in the pH range over which swelling changes occur. Second, the ultimate swelling ratio at high pH shifts downwards as temperature is raised. These shifts are consistent with increased hydrophobicity with increasing temperature, as expected based on the lower critical temperature (LCT) nature of the swelling transition of NIPA copolymer hydrogels.[24] The third trend is a sharpening of the swelling transition, particularly for the P05 gels, with increasing temperature. It is likely that the LCT effect is weak or absent at 25 °C but strong at 37 °C in the P05 hydrogels. The LCT effect is strong at both temperatures in the B05 hydrogels. Data is not shown for the more hydrophilic

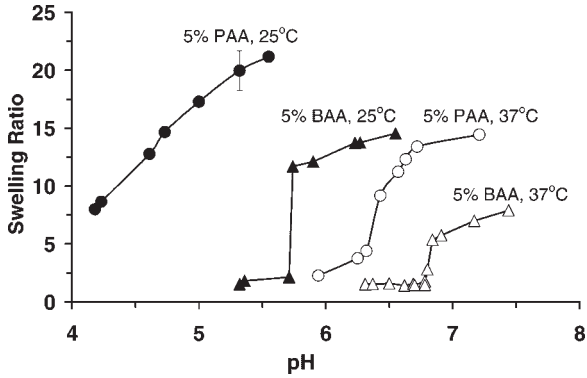

Figure 3.
pH-dependent equilibrium swelling curves at 25 °C and 37 °C for 5 mol% PAA (P05) and 5 mol% BAA (B05) hydrogel membranes.

copolymer hydrogels (A05, M05, and E05), as they did not show strong trends, since they were well below their respective LCT's at 25 °C.

pH oscillations

As described above, the oscillator depicted in Figure 1 operates due to nonlinear feedback between the pH-driven swelling

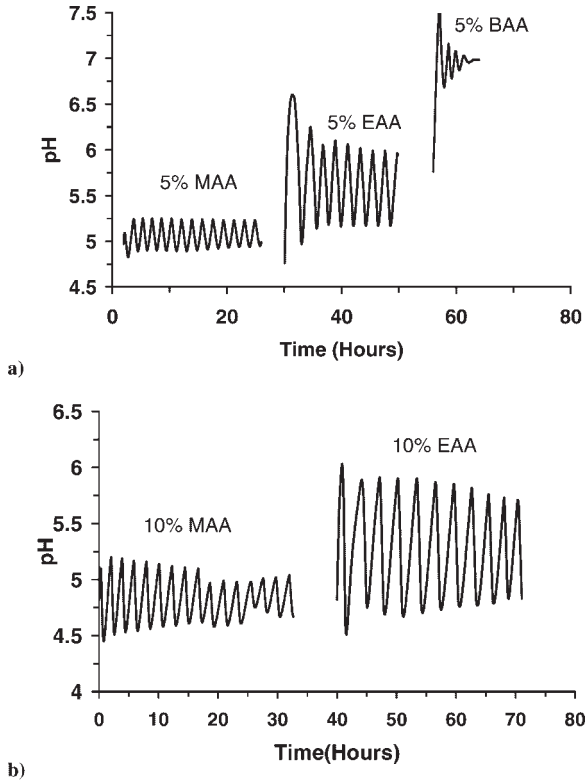

Figure 4.
pH oscillations in the receptor cell, observed with poly(NIPA-co-RAA) membranes included in the test cell of Figure 1. a) 5 mol% RAA (M05, E05 and B05 membranes); b) 10 mol% RAA (M10 and E10 membranes).

transition of the hydrogel membrane and the enzyme reaction converting glucose to H^+. As such, it is expected that a shift in VT pH will be reflected in a shift in pH range over which the system oscillates. This prediction is confirmed in Figure 4, for both R05 (Figure 4a) and R10 (Figure 4b) membranes. With increasing hydrophobicity of the membrane, the oscillations shifted in the alkaline direction, as did VT pH (Figure 2). Comparing Figure 4a and 4b, the effect of degree of substitution (R05 *versus* R10) is not so conclusive, however. As the effect of degree of substitution on VT pH was weaker than the effect of α-alkyl sidechain length, the latter observation is not surprising. It should also be noted that the concentration of glucose fed into the system varied from membrane to membrane in order to appropriately ''target'' the VT range. Because of this complication, it is difficult to quantitatively correlate VT pH with pH oscillation range. We conjecture that the decaying pH oscillations observed with the B05 membrane (Figure 4a) may be due to an inappropriate glucose feed concentration.

Conclusion

We have synthesized and characterized copolymer hydrogels based on NIPA and alkylacrylic acids for use in the drug delivery oscillator that is being developed for pulsatile delivery of GnRH. Significant shifts in VT pH in these gels were produced by copolymerizing NIPA with a range of higher-order homologs of acrylic acid. Alkaline shifts in the VT pH by more than 2 pH units could be engineered by manipulating the type and amount of the hydrophobic acid component incorporated into the hydrogel. This manipulation of hydrogel content also resulted in an alkaline shift in range of pH oscillations observed in the prototype drug delivery oscillator. Hydrophobicity of the polymer is therefore a useful control variable in determing the pH operating range of the drug delivery device.

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[1] B. Erman, P. J. Flory, *Macromolecules* **1986**, *19*, 2342.
[2] S. Hirotsu, *Adv. Poly. Sci.* **1993**, *110*, 1.
[3] S. Saito, M. Konno, H. Inomata, *Adv. Poly. Sci.* **1993**, *109*, 207.
[4] M. Shibayama, T. Tanaka, *Adv. Poly. Sci.* **1993**, *109*, 1.
[5] C. S. Brazel, N. A. Peppas, *Materials Research Society Symposium Proceedings* **1994**, *331*, 211.
[6] T. V. Burova, N. V. Grinberg, A. S. Dubovik, K. Tanaka, V. Y. Grinberg, A. Y. Grosberg, *Macromolecules* **2003**, *36*, 9115.
[7] M. B. Huglin, Y. Liu, J. L. Velada, *Polymer* **1997**, *38*, 5785.
[8] Z. Megeed, H. Ghandehari, *Polymeric Gene Delivery* **2005**, 489.
[9] M. E. Byrne, K. Park, N. A. Peppas, *Adv. Drug Deliv. Revs.* **2002**, *54*, 149.
[10] Y. Qiu, K. Park, *Adv. Drug Deliv. Revs.* **2001**, *53*, 321.
[11] S. H. Gehrke, N. R. Vaid, J. F. McBride, *Biotechnol. and Bioeng.* **1998**, *58*, 416.
[12] N. A. Peppas, J. Z. Hilt, A. Khademhosseini, R. Langer, *Adv. Mater.* **2006**, *18*, 1345.
[13] E. Ayano, K. Nambu, C. Sakamoto, H. Kanazawa, A. Kikuchi, T. Okano, *J. Chromatog., A* **2006**, *1119*, 58.
[14] G. P. Misra, R. A. Siegel, *J. Controlled Release* **2002**, *81*, 1.
[15] A. P. Dhanarajan, University of Minnesota (Twin Cities), **2003**.
[16] A. P. Dhanarajan, G. P. Misra, R. A. Siegel, *Journal of Physical Chemistry Part B* **2002**, *106*, 8835.
[17] A. P. Dhanarajan, J. Urban, R. Siegel, J. , Pojman, Q. Tran-Cong-Miyata, Eds., American Chmical Society, Washington DC **2004**, p. 44.
[18] J. P. Baker, R. A. Siegel, *Macromolecules* **1996**, *17*, 409.
[19] G. Misra, R. Siegel, *Journal of Controlled Release* **2002**, *81*, 1.
[20] A. Dhanarajan, J. Urban, R. Siegel, in: ''*Nonlinear Dynamics in Polymeric Systems*'', J. Pojman, T.-C.-M. Qui, Eds., Oxford University Press, Washington, DC **2004**.
[21] P. J. Flory, ''*Principles of Polymer Chemistry*'', Cornell University Press, Ithaca, New York **1953**.
[22] M. Ferrito, D. A. Tirrell, *Macromol. Synth.* **1992**, *11*, 59.
[23] ''*Dissociation Constants of Organic Acids in Aqueous Solutions*'', 55th edition, CRC Press, Cleveland **1974**.
[24] Y. Maeda, T. Nakamura, I. Ikeda, *Macromolecules* **2001**, *34*, 8246.

