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Controlled release of 9-β-D-arabinofuranosyladenine from thermo-responsive devices based on acryloyl-L-proline methyl ester

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Summary

Loosely cross-linked polymer gels consisting of acryloyl-L-proline methyl ester (A-ProOMe), 2-hydroxyethyl methacrylate (HEMA), and polyethylene glycol dimethacrylate (*n*G) were synthesized by radiation-induced polymerization, using γ-rays from a ^{60}Co source. The percent swelling behavior of the gels obtained was examined in water in the range of 0–50°C, showing that gels exhibit a characteristic thermo-response such as low-temperature swelling and high-temperature deswelling, depending strongly upon monomer composition. 9-β-D-Arabinofuranosyladenine was incorporated into this polymer gel to evaluate the pulsatile drug release when cycled at 10 and 37°C, in which the drug release kinetics could be explained by a matrix pumping mechanism.

Introduction

A large number of studies have been conducted in order to design controlled-release drug delivery systems. The majority of such investigations were aimed at obtaining a constant release of drug from the system, however, in an ideal drug delivery system the drug should be released depending on the condition of disease. In order to design such a drug delivery system, the development of 'intelligent' polymers that respond to environmental changes, for example, in pH, chemical, temperature, and electric field, is of the utmost importance. For this purpose, hydrogels have been widely investigated as suitable materials due to their high hydrophilicity, flexibility,

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ease of shaping treatment, ease of swelling control, and ease of drug release control. For this purpose, thermo-responsive poly(*N*-isopropylacrylamide) gel has intensively investigated, e.g., for immobilization of enzymes (Dong et al., 1986), therapeutics and diagonistics (Hoffman, 1987), drug delivery (Dong et al., 1990), and controlled insulin delivery (Bae et al., 1989).

It is well-known that 9-β-D-arabinofuranosyladenine (Ara-A) exhibits significant antiviral activity against herpes viruses replicating in cell cultures (Miller et al., 1969), animals (Schabel et al., 1968; Kaufman et al., 1970), and humans (Hyndiuk et al., 1975; McKinnon et al., 1975). Ara-A is subject to metabolism for a short time after percutaneous absorption and a resulting significant efficacy for herpes therapy cannot be expected. In order to maintain the efficacy of the drug over a relatively long period, it should be incorporated into the transdermal membrane with controlled release of Ara-A. Initially, we reported the permeation of Ara-A from gel membranes consisting of poly(2-hydroxyethyl methacrylate) (HEMA), poly(HEMA-co-styrene), and poly(HEMA-co-*N*-vinyl-2-pyrrolidone) and proved that the blood level of Ara-A could be controlled by changing the degree of hydration of the membranes when they were used as transdermal devices in hairless mice (Miyajima et al., 1987; Komada et al., 1991). However, such membranes lack any function that responds to environmental changes. Recently, we synthesized new external-stimulus-responsive gels with high biocompatibility, using acryloyl derivatives with pendant α-amino acids, e.g., acryloyl-L-proline methyl ester (A-ProOMe) (Yoshida et al., 1991). This gel exhibits a lower critical solution temperature (LCST) of 14°C in water (Yoshida et al., 1992). In the present paper, the A-ProOMe monomer was copolymerized with a hydrophilic HEMA monomer in the presence of a small amount of cross-linking agents by radiation-induced polymerization, in order to evaluate the swelling-deswelling behavior of gels around the LCST temperature. In addition, Ara-A was loaded into this gel to estimate the in vitro drug delivery from a thermo-responsive specimen, which can be applied as an intelligent transdermal device.

Materials and Methods

Materials

Acryloyl-L-proline methyl ester (A-ProOMe) was synthesized by condensation of acrylic acid and L-proline methyl ester hydrochloride in the presence of dicyclohexylcarbodiimide as a condensing agent as described previously (Yoshida et al., 1991); $R_f = 0.54$ (ethyl acetate, Merck kieselgel $60F_{254}$ plate), $[\alpha]_D = -133.7°$ ($c = 1$ wt% in methanol at 28°C). HEMA and a series of polyethylene glycol dimethacrylate (*n*G = 2G, 4G, 9G, 14G, and 23G) were purchased from Shin-Nakamura Chemical Co. (Osaka, Japan). 9-β-D-Arabinofuranosyladenine (Ara-A), which has the structural formula depicted in Fig. 1, was obtained from Sigma Chemical Co.

Preparation of thermo-responsive gels

A mixture of 10 mmol of A-ProOMe and HEMA of the desired compositions and 1 ml of ethanol containing 0.02 mmol of cross-linking agents were charged into an 8 mm internal diameter glass ampoule with a flat bottom. The ampoule was sealed under nitrogen gas and then irradiated for 3 h at a dose rate of 10 kGy/h at 25°C using γ-rays from a ^{60}Co source. After irradiation, the solid and transparent polymer gels were obtained in rod-like form by separating the product from the ampoule. This product was washed with excess ethanol at 25°C to remove unreacted monomer. The polymer yield, which was defined as the ethanol-insoluble gel, was 94–97%. The gels obtained were allowed to swell in distilled-deionized water at 0°C, then lyophilized, and reswollen in water at 0°C for 3 weeks. The structural formulae of gels consisting of A-ProOMe, HEMA, and *n*G (2G, diethylene

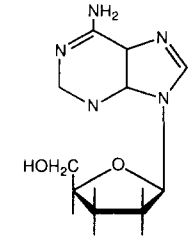

Fig. 1. Structural formula of Ara-A.

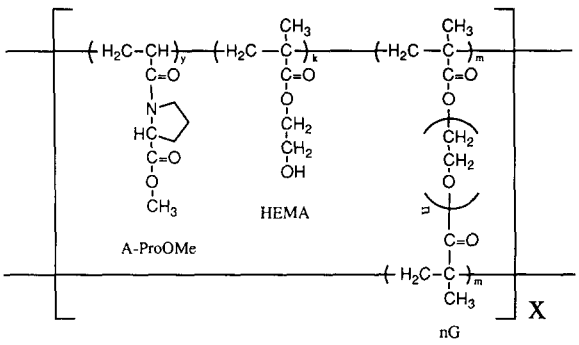

Fig. 2. Structural formulae of polymer hydrogels consisting of A-ProOMe, HEMA, and *n*G.

glycol dimethacrylate; 4G, tetraethylene glycol dimethacrylate; 9G, nonaethylene glycol dimethacrylate; 14G, tetradecaethylene glycol dimethacrylate; 23G, tricosaethylene glycol dimethacrylate) are shown in Fig. 2.

Swelling of thermo-responsive gels

The loosely cross-linked gels were previously swollen in water at 0°C until equilibrium was reached. The percent swelling (water absorption) of the gel was estimated according to the following equation: swelling $(\%) = 100(W - W_0)/W_0$, where W is the weight of the swollen gel and W_0 the weight of the dried gel.

Loading of Ara-A in thermo-responsive gels

Ara-A was saturated with a 10:90 vol.% water/methanol mixture at 10°C. The lyophilized gel was immersed in this saturated solution at 10°C until equilibrium was attained. The weight of drug loaded in the gel was estimated from the weights of gels before and after loading.

The in vitro release of Ara-A from thermo-responsive gels was determined in water by repeating measurements between 10 and 37°C at 60-min intervals. The amount of drug released was assayed at 259 nm using a Hitachi U-3210 spectrophotometer.

Microscopic observation

The appearances of gels after swelling and deswelling in water at different temperatures were observed with a Jeol JXA-733 scanning electron microscope (SEM). For this purpose, the treated gel was immediately immersed in liquid nitrogen (−196°C), then lyophilized, and observed microscopically.

Results and Discussion

The temperature dependence of the percent swelling of loosely cross-linked poly(A-ProOMe-co-HEMA) gels in water is shown in Fig. 3 as a function of monomer composition. The LCST temperature of homopoly(A-ProOMe) was approx. 14°C in water. In a loosely cross-linked poly(A-ProOMe) gel, the swelling-deswelling kinetic process occurred discontinuously around the LCST temperature, leading to a characteristic thermo-response such as low-temperature swelling and high-temperature deswelling. However, this response is markedly influenced by the introduction of the HEMA moiety, in which the poly(A-ProOMe-co-HEMA) gel exhibited continuous shrinkage with rising temperature up to

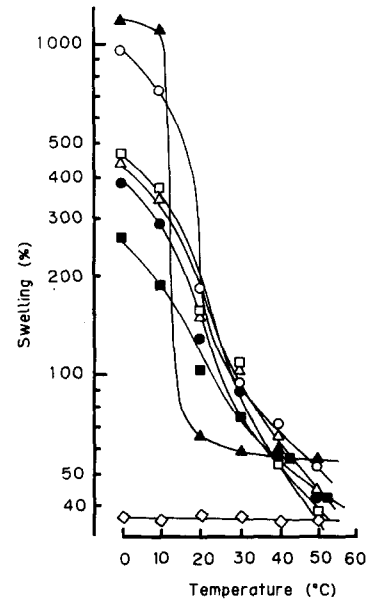

Fig. 3. Temperature dependence of percent swelling of poly(A-ProOMe-co-HEMA-co-9G) gels with compositions of (▲) 10:0:0.02 mmol, (○) 9.5:0.5:0.02 mmol, (□) 9:1:0.02 mmol, (△) 8:2:0.02 mmol, (●) 7:3:0.02 mmol, (■) 5:5:0.02 mmol and (◇) 0:10:0.02 mmol. The gels, which were previously treated with water at 0°C for 3 weeks, were incubated at each temperature for 24 h.

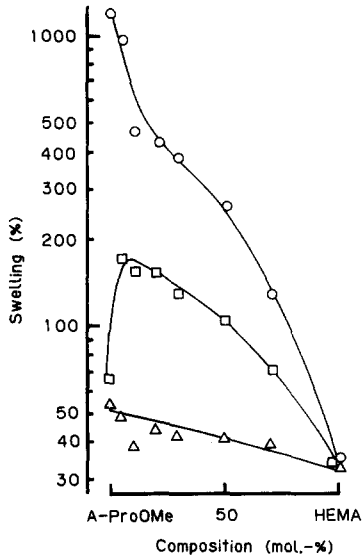

Fig. 4. Effect of monomer composition on the percent swelling of polymer gels consisting of 10 mmol of a mixture of A-ProOMe and HEMA and 0.02 mmol of 9G as a cross-linking agent. The gels, which were previously treated with water at 0°C for 3 weeks, were incubated at temperatures of (○) 0°C, (□) 20°C, and (△) 50°C for 24 h.

50°C after showing the greatest degree of swelling at 0°C. On the basis of the data in Fig. 3, the data on the relationship between the gel swelling and the A-ProOMe/HEMA composition were replotted and are shown in Fig. 4 as a function of temperature. The introduction of the HEMA moiety into the copolymer markedly retards not only the degree of gel swelling at 0°C but also that of gel shrinkage at 20°C. The resulting swelling-deswelling behavior disappeared completely for a loosely cross-linked poly(HEMA) gel. The effect of number of the ethylene glycol $[(CH_2CH_2O)_n]$ units in the cross-linking nG agent on the percent swelling of poly(A-ProOMe-co-HEMA-co-nG, 9:1:0.02 mmol) gels is shown in Fig. 5. The hydrophilicity of cross-linking agents increases with increasing number of ethylene glycol units in nG. In a strongly hydrophobic 2G-containing gel system, the percent swelling was relatively high, compared with that of other nG-containing gel systems, but no effect was observed with gels involving cross-linking agents < 4G.

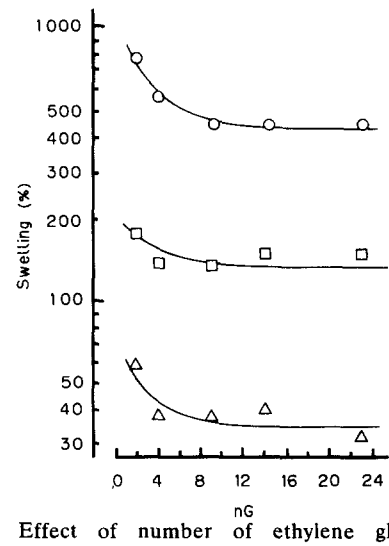

Fig. 5. Effect of number of ethylene glycol units $[(CH_2CH_2O)_n]$ in nG monomer on the percent swelling of poly(A-ProOMe-co-HEMA-co-nG, 9:1:0.02 mmol) gels. The gels, which were previously treated with water at 0°C for 3 weeks, were incubated at temperatures of (○) 0°C, (□) 20°C, and (△) 50°C for 24 h.

The changes in percent swelling and deswelling with passage of time were examined for a poly(A-ProOMe-co-HEMA-co-9G, 9 : 1 : 0.02

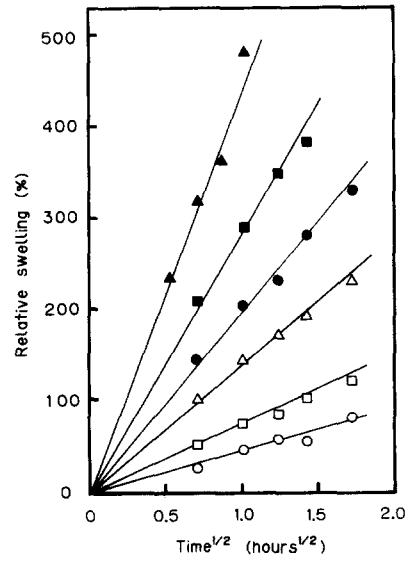

Fig. 6. Deswelling behavior of a poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gel when treated at temperatures of (○) 5, (□) 10, (△) 25, (●) 30, (■) 40, and (▲) 50°C after preswelling with water at 0°C for 3 weeks.

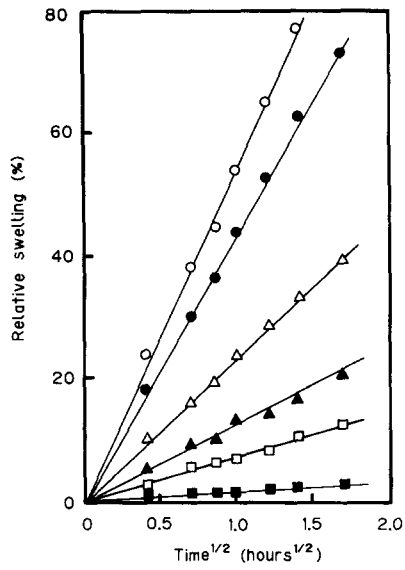

Fig. 7. Swelling behavior of a poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gel when treated at temperatures of (○) 5, (●) 10, (△) 15, (▲) 20, (□) 30, and (■) 40°C after preswelling with water at 50°C for 3 days.

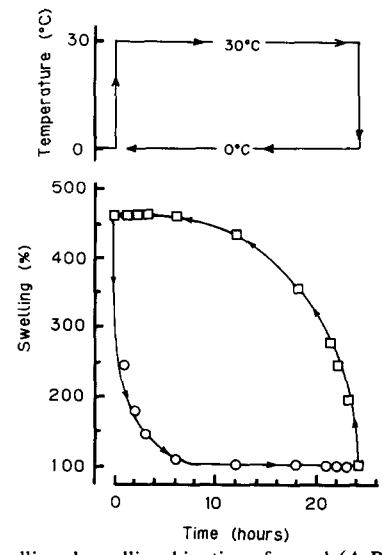

Fig. 8. Swelling-deswelling kinetics of a poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gel between 0 and 30°C in water.

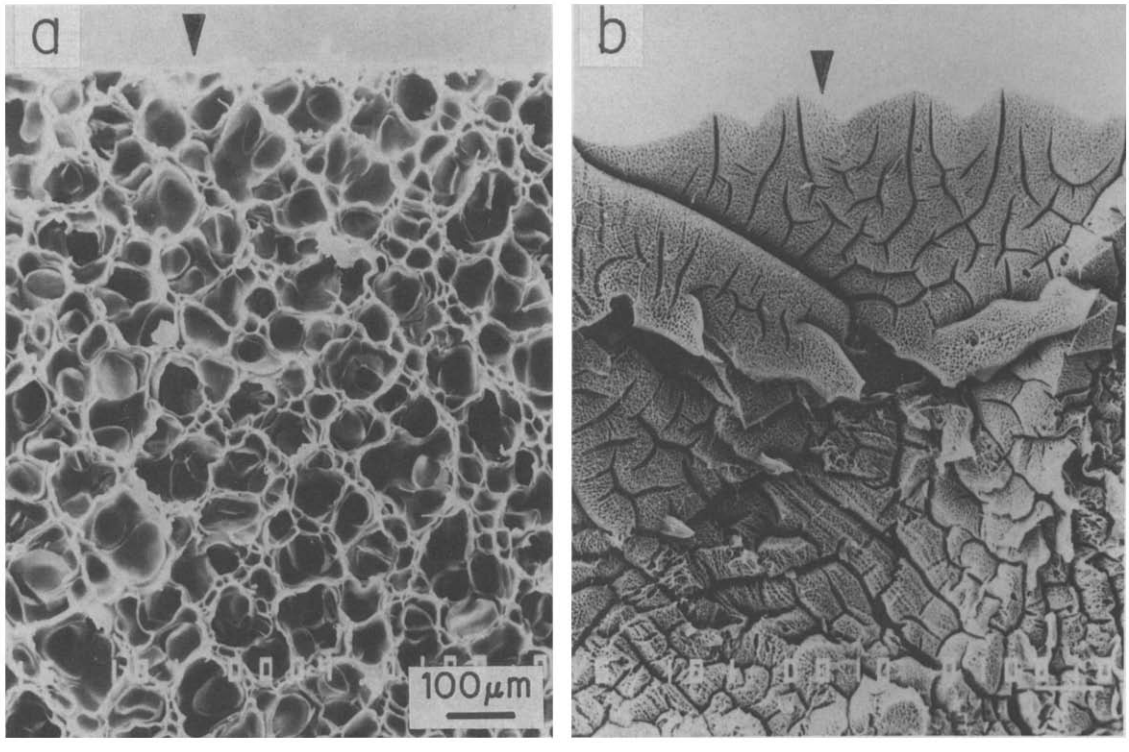

Fig. 9. SEM photographs of cross-sectional poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gels when treated at temperatures of (a) 0°C and (b) 30°C for 30 min after preswelling with water at 0°C for 3 weeks. Arrows indicate the surface of gels.

mmol) gel. In deswelling, the gel was treated at temperatures of 5, 10, 15, 20, 25, 30, 40, and 50°C after preswelling with water at 0°C for 3 weeks, as seen in Fig. 6. On the other hand, in swelling, the gel was previously shrunken at 50°C for 3 days, followed by a swelling treatment at temperatures of 5, 10, 15, 20, 30, and 40°C. This result is illustrated in Fig. 7. From a comparison of Figs 6 and 7, it was found that the rate of gel deswelling is much faster than that of gel swelling. In order to clarify this difference, the rate constant (k) was estimated from the slope of the linear portion of the plot of changes in deswelling and swelling against the square root of time. The k values for gel deswelling were found to be $44.4\%/h^{1/2}$ at 5°C, $60.5\%/h^{1/2}$ at 10°C, $62.5\%/h^{1/2}$ at 15°C, $72.3\%/h^{1/2}$, $132.9\%/h^{1/2}$ at 25°C, $192.7\%/h^{1/2}$ at 30°C, $300.0\%/h^{1/2}$ at 40°C, and $511.1\%/h^{1/2}$ at 50°C, in contrast to $55.2\%/h^{1/2}$ at 5°C, $46.0\%/h^{1/2}$ at 10°C, 2[illegible]

$6.2\%/h^{1/2}$ at 30°C, and $2.6\%/h^{1/2}$ at 40°C for the gel swelling.

The swelling-deswelling kinetics of a poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gel when cycled between 0 and 30°C at 24-h intervals are shown in Fig. 8. On immersion of the gel preswollen at 0°C in water kept at 30°C, rapid shrinkage took place until equilibrium was reached after 8 h. In contrast, in gel reswelling, the periods required for saturation of swelling were approx. 18 h. It was confirmed that the swelling-deswelling kinetics at 24-h intervals are reversible throughout an experimental period of 720 h. The appearance of the gel was translucent in the swollen state at 0°C, however, this changed to become opaque as a result of the rapid shrinkage of the whole gel at 30°C. In order to elucidate this phenomenon, the cross-sectional and interior structures of gels were observed microscopically and are shown in Figs 9 and 10. The gel at 0°C

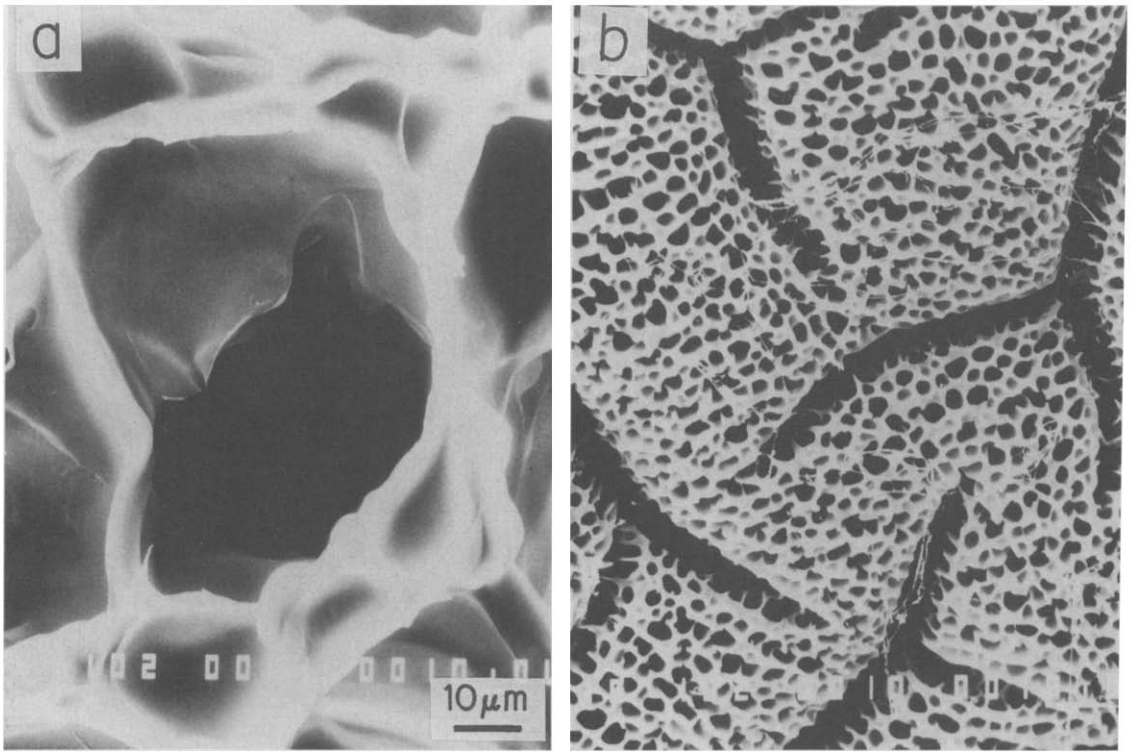

Fig. 10. SEM photographs of interior poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gels. Experimental conditions and symbols are the same as those in Fig. 9.

(Figs 9a and 10a). However, both pore and gel sizes were markedly reduced on treatment at 30°C (Figs 9b and 10b). In this case, the disappearance of pores was observed neither at the surface in contact with water nor in an interior matrix even in the deswollen state. Therefore, it is reasonable to consider here that the rapid deswelling of the gel is caused by the rapid passage of water throughout the porous structure as a result of processes such as pumping and shrinkage, whilst the slow swelling is due to the penetration of water into the shrunken gel being hindered owing to the formation of highly rigid membrane wall. The difference in activation energies between gel swelling and deswelling may also be related to the ease with which water diffuses throughout the porous structure of the gel.

Ara-A was incorporated into a poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gel with matrix pumping function to evaluate the in vitro release behavior. The gel employed, which has a size of 8 mm in diameter and 5 mm in length, involves approx. 4.5 mg of Ara-A. The in vitro dose of Ara-A released from the gel was found to occur in the form of unstable release accompanying an initial burst during the first 24 h period, followed by a constant release of approx. 11 ng/h at 10°C for 9 days from day 1 to 10. On the basis of this result, we decided to pretreat the drug-loaded gel in water at 10°C during the first 24 h period in order to avoid the unstable period of release. Using this pretreated gel, the in vitro release behavior of Ara-A from the gel when cycled between 10 and 37°C at 60-min intervals was examined and is depicted in Fig. 11. The in vitro release rate of Ara-A from the gel treated for 60 min at 10°C was approx. 11 ng/h, in contrast to approx. 33 ng/h at 37°C, suggesting that the release behavior of drug when cycled at different temperatures is reversible. The cause of the high release rate of Ara-A at 37°C could readily be explained on the basis of the 'matrix pumping' mechanism owing to the shrinkage of the gel. On the other hand, the diffusion of water from the surface of the shrunken gel to its interior may play an important role in the low level of drug release at 10°C.

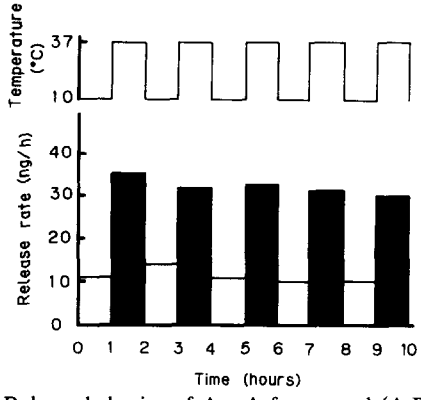

Fig. 11. Release behavior of Ara-A from a poly(A-ProOMe-co-HEMA-co-9G, 9:1:0.02 mmol) gel when cycled at 10 and 37°C at 60-min intervals.

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