Thermoresponsive release from poly(L-lactic acid) microcapsules containing poly(*N*-isopropylacrylamide) gel

Tongjit Kidchob, Shunsaku Kimura and Yukio Imanishi

Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto, 606-01, Japan

Many internal cavities of poly(L-lactic acid) (PLLA) microcapsules have been filled partly or completely with poly(*N*-isopropylacrylamide) (PNIPAAm) gel by two different procedures to obtain microcapsules for thermoresponsive drug release. In one procedure, the PLLA microcapsules have been incubated with *N*-isopropylacrylamide (NIPAAm) for different hours, and the polymerization has been carried out by using a redox initiator. The longer the incubation time, the more PNIPAAm gel is loaded. In the other procedure, the internal cavities of PLLA microcapsules fully loaded with PNIPAAm gel have been prepared by evaporation of a w/o/w emulsion after the polymerization of NIPAAm contained in the internal aqueous phase. Dextran as a model drug has been loaded in the microcapsules, and the release has been examined at two different temperatures which are above or below the lower critical solution temperature (LCST) of PNIPAAm. The dextran release is accelerated above LCST, and nearly stops below LCST. The release rate of dextran is dependent on the amount of PNIPAAm gel loaded in the microcapsules as well as on the size of the internal cavities which is controllable by the preparation method of w/o emulsion.

Introduction

Intelligent materials change their properties in response to external conditions such as pH change,[1,2] temperature change,[3–6] photoirradiation[7] and the nature of ionic species.[8] Recently, an increasing number of reports have been published on intelligent materials for drug delivery systems. For example, poly(*N*-isopropylacrylamide) (PNIPAAm) and its copolymers were used as the key polymer for temperature-dependent drug release.[3–6]

PNIPAAm possesses a lower critical solution temperature (LCST) around 32 °C, and a PNIPAAm gel reversibly swells below the LCST and shrinks above the LCST.[9–11] Consequently, the PNIPAAm gel loading drug shows different behaviors of drug release depending on the temperature. A disk of PNIPAAm copolymer gel released drug rapidly at low temperature, but slowly at high temperature (negative thermoresponse).[5] However, when the gel was put in a 'Teflon' capsule with a small aperture, a positive thermoresponse was observed.[6] There have also been several investigations to improve the responsiveness of gel by preparing 'macroporous' hydrogel or hydrogel carrying PNIPAAm grafts.[12,13]

Hydrogel is weak in physical strength which limits applications to the drug delivery system. However, microcapsules have enough strength for the application, and also protect water-soluble substances from degradation and release drugs at a suitable rate. Therefore, the weakness of the hydrogel can be overcome by encapsulation in microcapsules. Poly(L-lactic acid) (PLLA) was used in the present study for the microcapsule preparation, because PLLA microcapsules were biodegradable and frequently used for drug delivery systems. In addition, PLLA microcapsules, which were prepared from water/oil/water (w/o/w) emulsion by the solvent evaporation method, are known to possess the honeycomb-type structure.[14,15] Internal cavities of PLLA microcapsules were filled, here, partly or completely with PNIPAAm gel. The gel was encapsulated in small internal cavities with small apertures, expecting a positive thermoresponsive release. Distribution of PNIPAAm gels to many small cavities brought about a huge total surface area of the gel. This situation may lead to a fast response. The size of internal cavities depends on the method of mixing, magnetic stirring or vortex mixing, in the preparation of a w/o emulsion. Dextran was encapsulated as a drug model, and the release was studied at different temperatures to investigate the effect of the size of internal cavities to which gels are loaded and the amount of gel loaded.

Experimental

Materials

PLLA was synthesized by ring-opening polymerization of L-lactide (Shimadzu, Co., Japan) with stannous octanoate as a catalyst. The stannous octanoate was a kind gift from Professor Yoshiharu Kimura, Kyoto Institute of Technology. The molecular weight of the obtained PLLA was determined as 60 000 by GPC measurement with polystyrene as standard. *N*-isopropylacrylamide (NIPAAm) (Eastman Kodak, Co., USA) was recrystallized from hexane. *N*,*N*′-Methylenebis-(acrylamide) (MBAAm; Nacalai Tesque, Inc., Japan) was recrystallized from ethanol. Ammonium peroxodisulfate (AP, Wako Pure Chemicals, Inc., Japan) was used as a redox initiator with tetramethylethylenediamine (TEMED). Gelatin (type B, from bovine skin) and fluorescein-isothiocyanate (FITC)-labeled dextran (weight-average molecular weight of 72 000) were purchased from Sigma Chemical Co., USA. Poly(vinyl alcohol) (PVA, weight-average molecular weight of 85 000–145 000, degree of saponification of 87–89 mol%) was purchased from Aldrich Chemical Co., USA.

Preparation of microcapsules

Microcapsules used in the present investigation were prepared from w/o/w emulsion by the solvent-evaporation method. Briefly, an aqueous solution (1.1 ml) of FITC-dextran (15 mg) and gelatin (33 mg) were added to a chloroform solution (5.0 ml) of PLLA (375 mg). The w/o emulsion was prepared by magnetic stirring for 10 min or by vortex mixing for 3 min. Then the emulsion was added to an aqueous solution (80 ml) of PVA (0.80 g) under stirring to form a w/o/w emulsion. Stirring was continued at room temperature for 30 min and at 40 °C for 3 h to evaporate chloroform completely. Microcapsules were separated by sieving through a mesh of 212–425 μm and washed several times with distilled water.

Preparation of microcapsules containing PNIPAAm gel in the cavities in the surface region (method 1)

The microcapsules (150 mg) were incubated in an aqueous solution (2 ml) containing NIPAAm (20 wt%) and MBAAm (1 wt%) for 8 or 48 h. The dispersion was centrifuged at 200 g, and the microcapsules were resuspended in water and bubbled with nitrogen gas. An aqueous solution (2 ml) containing ammonium peroxodisulfate (3.0 mg) and tetramethylethylenediamine (9.0 μl) was then added to initiate the polymerization of NIPAAm infiltrated into the microcapsules. After 24 h, the microcapsules were repeatedly washed with deionized water. The microcapsules were immediately used for the release experiments of fluorescein-labelled (FITC)-dextran under various conditions.

Preparation of microcapsules containing PNIPAAm gel in all internal cavities (method 2)

An aqueous solution (1.2 ml) containing NIPAAm (10 wt%), MBAAm (1 wt%) and FITC-dextran (4 wt%) was added to a chloroform solution (5 ml) of PLLA (375 mg) containing span 60 (3 wt%). The solution was bubbled with nitrogen gas for 15 min. An aqueous solution (100 μl) containing ammonium peroxodisulfate (1.0 mg) and tetramethylethylenediamine (TEMED, 3.0 μl) was added to the microcapsule dispersion to obtain w/o emulsion by magnetic stirring for 10 min or by vortex mixing for 3 min. Then the emulsion was added to an aqueous solution (80 ml) of PVA (0.80 g) under stirring to form a w/o/w emulsion. After polymerization and solvent evaporation over 24 h, the microcapsules were separated and washed several times with water. The microcapsules were immediately used for release experiments of FITC-dextran under various conditions.

Determination of the amount of PNIPAAm gel in microcapsules

The amounts of PNIPAAm encapsulated in the obtained microcapsules were estimated by FTIR spectroscopy (a Nicolet Magna 850 Fourier transform infrared spectrophotometer, USA) from the increase in the peak area assigned to the amide II absorption (1525 cm^{-1}) by taking the ester carbonyl absorption (1750 cm^{-1}) of PLLA as standard. Before the determination analysis, FITC-dextran, the remaining NIPAAm oligomers and unreacted NIPAAm were removed out from the microcapsules by washing with distilled water under sonication for 10 min. The suspension was centrifuged and the supernatant was discarded. These processes were repeated at least 5 times, and the samples, which were dried under vacuum for 24 h, were subjected to FTIR measurement by a KBr disk method.

Morphology of microcapsules

Microcapsules suspended in water were freeze-dried. Then, the dried microcapsules were mounted on metal stubs with a double-side tape and coated with gold layer using an ion-coater (Eiko IB-3, Tokyo, Japan). The surface of the microcapsules was observed by a Hitachi S-510 Scanning Electron Microscope (SEM, Tokyo, Japan).

Release experiment

The microcapsules obtained (25 mg) were suspended in a Tris buffer solution (0.05 M, pH 7.5, 10 ml), which was shaken in a thermostatted water bath. The temperature of the suspension was varied between 25 °C (below the LCST of PNIPAAm) and 40 °C (above the LCST of PNIPAAm) at a short interval of 1.5 or 2.0 h and at a long interval of 12 h. In the former experiment, the suspension (2 ml) was withdrawn every 30 min and centrifuged at 200 g, and the supernatant was subjected to fluorescence spectroscopy to determine the concentration of FITC-dextran released into solution. Excitation and monitoring wavelengths were 470 and 520 nm, respectively. A buffer solution (2 ml) was supplied to the suspension to keep the total volume of the suspension at 10 ml during the incubation. In the latter experiment, every 12 h the temperature was changed and the concentration of FITC-dextran released into solution was determined by a similar method to that described above. These procedures were repeated, and the microcapsules were finally destroyed by the addition of chloroform (1 ml) and sonication for 3 min. Then the suspension was centrifuged at 1600 g for 10 min and the supernatant was subjected to fluorescence spectroscopy to determine the FITC-dextran remaining in the microcapsules. Each experiment was done in duplicate and the data were averaged.

Results and discussion

Preparation of microcapsules

The method used for preparation of the microcapsules is illustrated in Scheme 1. In total, eight types of microcapsules were prepared. Four kinds of microcapsules were prepared by magnetic stirring in the preparation of a w/o emulsion (MM, MM8, MM48 and MMG) and the rest by vortex mixing (MV, MV8, MV48 and MVG). The numerical numbers in the symbol represent the incubation time of microcapsules with monomer solution. MM and MV are microcapsules which do not contain PNIPAAm gel. PNIPAAm gel was encapsulated in the MV8, MV48, MM8 and MM48 microcapsules by method 1, and in the MMG and MVG microcapsules by method 2.

The scanning electron micrographs (SEM) of the microcapsules MV, MV8, MV48 and MVG are shown in Fig. 1. There is little difference in the surface images of the MV, MV8 and MV48 microcapsules. In contrast, several large pores are seen on the surface of the MVG microcapsule. Since the microcapsule membrane and PNIPAAm gel were formed simultaneously in the MVG microcapsule, the membrane may not be mechanically strong enough to hold the gel, resulting in the formation of large pores on the surface. Similarly, the MMG microcapsules had cracks on the membrane surface, but MM, MM8 and MM48 microcapsules did not.

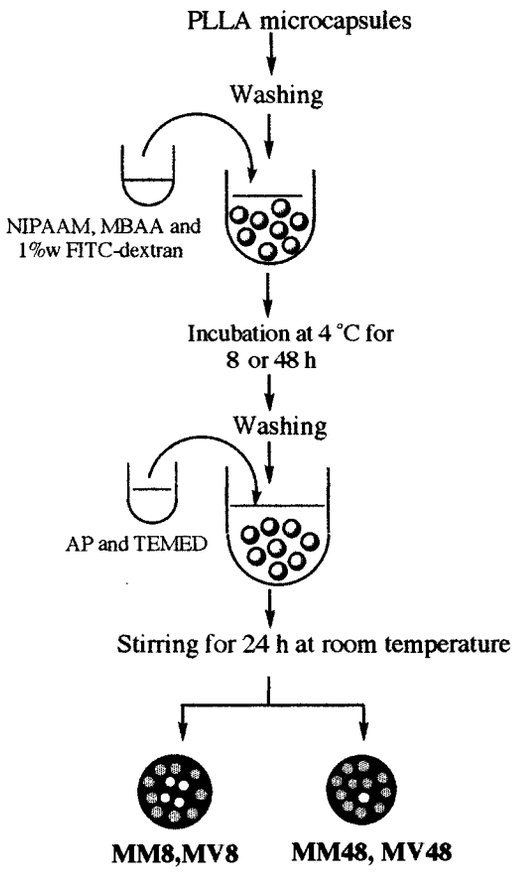
The SEM of the microcapsule cross-section is shown in Fig. 2. A number of small cavities are seen in each microcapsule irrespective of the method, magnetic stirring or vortex mixing, employed for the w/o emulsion preparation. However, the size of cavities was 5–25 μm for the magnetic-stirring microcapsules and 2–10 μm for the vortex-mixing microcapsules. The pore diameter on the microcapsule surface was 0.5–3.0 and 0.1–1.0 μm for those prepared by the magnetic-stirring and the vortex-mixing method, respectively.

The spatial distribution of PNIPAAm gel in the microcapsule is such that the density near the surface should be higher than the center part of the microcapsule which was prepared by method 1. The microcapsule was prepared first and was then incubated with a NIPAAm solution. Therefore, a gradient of NIPAAm concentration from the surface to the center of the microcapsule should be formed, and this gradient should reflect the gradient of PNIPAAm gel in the microcapsule. In addition, the longer the incubation time was, the more PNIPAAm gel was loaded in the microcapsule as explained in the next section.

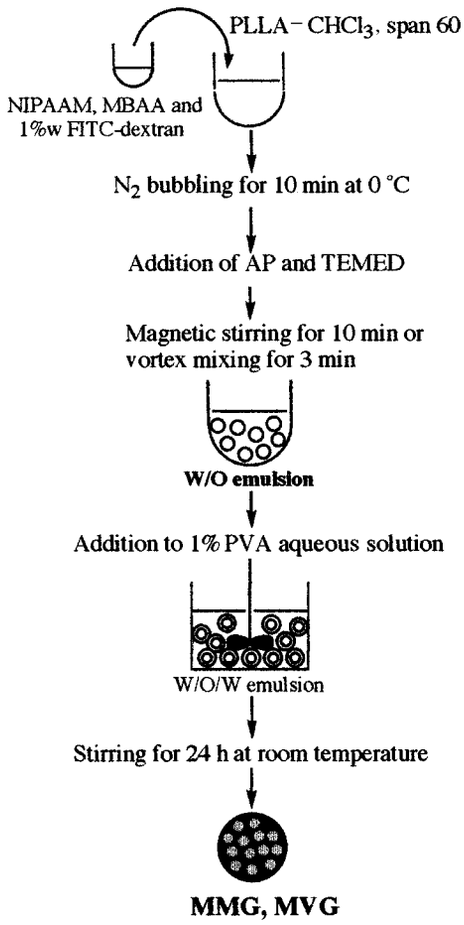
Amount of PNIPAAm gel loaded in the microcapsules

Fig. 3 shows the IR spectra of the microcapsules prepared by vortex mixing. The ester carbonyl absorption (1750 cm^{-1}) of PLLA and the amide I absorption (1650 cm^{-1}) and amide II absorption (1525 cm^{-1}) of PNIPAAm are observed. The relative intensity of the amide II absorption compared with the ester carbonyl absorption increased in the order of MV8 < MV48 < MVG and MM8 < MM48 < MMG (data not shown). The amide I absorption was not suitable for the estimation because the absorption band overlapped with the water absorption band. The amount of PNIPAAm gel loaded in those microcapsules was then estimated from the peak area of amide II absorption against the constant value of ester carbonyl

Preparation of PLLA microcapsules containing PNIPAAm gel in the internal cavities in the surface region



Preparation of PLLA microcapsules containing PNIPAAm gel in all the internal cavities



Scheme 1 Schematic illustration of PLLA microcapsules containing PNIPAAm gel in the internal cavities in the surface region and those containing PNIPAAm gel in all the internal cavities

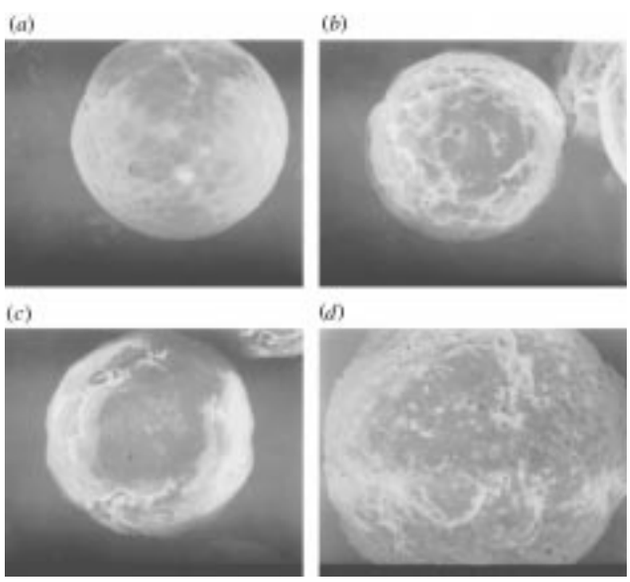


Fig. 1 Scanning electron micrographs of PLLA microcapsules prepared by vortex mixing (*a*) MV (× 600); those containing PNIPAAm gel in the surface cavities, (*b*) MV 8 (× 600) and (*c*) MV48 (× 600); that containing PNIPAAm gel in all the cavities, (*d*) MVG (× 500)

absorption, and the amount of PNIPAAm gel per 1 mg of PLLA was calculated to be 18, 32 and 78 μg for MV8, MV48 and MVG and 29, 60 and 99 μg for MM8, MM48 and MMG, respectively.

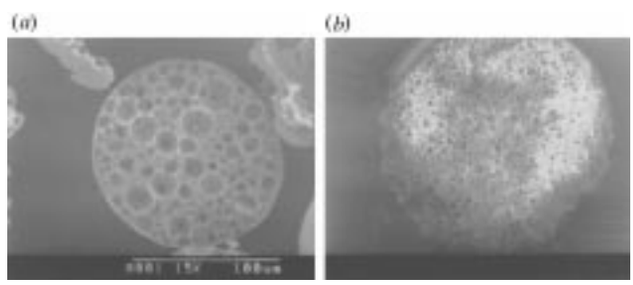


Fig. 2 Scanning electron micrographs (cross-section) of PLLA microcapsules prepared by (*a*) magnetic stirring (× 300) and (*b*) vortex mixing (× 500)

Temperature-dependent release

Fig. 4 shows the amount of FITC-dextran released from MM, MM8, MM48 and MMG microcapsules at regularly changing temperatures of 25 and 40 °C. With the microcapsule which does not contain PNIPAAm gel (MM), the release rate was relatively high at the beginning, but soon slowed down, because the amount of dextran remained in the microcapsules decreased. The release profile did not show a clear thermo-response. However, the microcapsules with PNIPAAm gel inside changed the release rate in response to temperature. Notably, the thermal response of the microcapsules is quick. This response may be due to the large surface area of PNIPAAm gel as a result of distribution to many small internal cavities.

The release rate became faster at 40 °C and nearly ceased at 25 °C. The very slow release at 25 °C may be due to the high

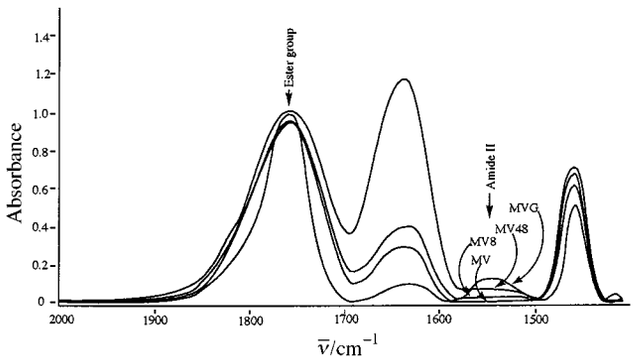


Fig. 3 IR spectra of MV, MV8, MV48 and MVG

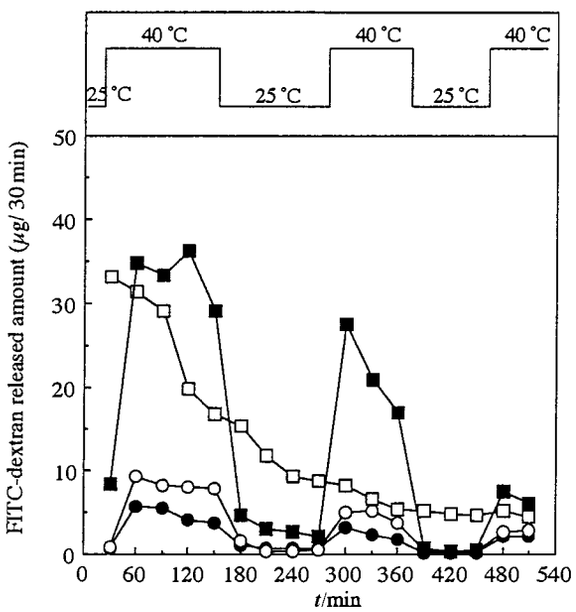


Fig. 4 Release rate of FITC-dextran released from the PNIPAAm-loaded microcapsule in response to the temperature change between 25 and 40 °C in a Tris buffer solution; MM (□), MM8 (●), MM48 (○) and MMG (■) microcapsules. Two sets of experiments were averaged where the difference was within 9%.

molecular weight of FITC-dextran which makes diffusion in the gel difficult. Therefore, the gel swollen in the internal cavities obstructs the release of drug through the aperture of the microcapsule membrane. When the temperature was raised to 40 °C, the PNIPAAm gel in the small cavities shrank and open-space was produced in the cavities, through which dextran diffuses freely. In addition, some dextran may be squeezed out from the gel. Thus, the released amount of dextran should increase at 40 °C.

MM48 released FITC-dextran faster than MM8, showing the temperature-dependent pattern of release. MMG showed even faster release than MM48, which is comparable to MM. It is therefore concluded that the release rate increases with the increasing amount of PNIPAAm gel loaded in the microcapsules. Therefore, the effect of the squeezing FITC-dextran from the gel due to shrinkage of PNIPAAm gel at 40 °C is significant.

The temperature effect on the FITC-dextran release from MV, MV8, MV48 and MVG microcapsules is shown in Fig. 5. The release pattern is as a whole very similar to that in the MM series. The release was fast at 40 °C but slowed down at 25 °C. The amount of the released dextran decreased monotonously with time when kept at constant temperature. When the temperature was changed repeatedly between 40 and 25 °C, the fast and slow releases were observed in response to the temperature.

The release becomes faster in the order of MV8 < MV48 < MVG < MV, which reflects an increasing order of the amount of PNIPAAm gel loaded in the microcapsules. However, the

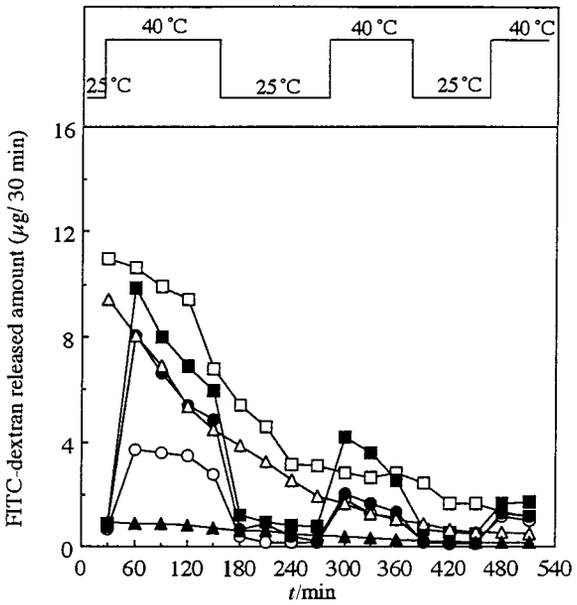


Fig. 5 Release rate of FITC-dextran released from the PNIPAAm-loaded microcapsule in response to temperature change between 25 and 40 °C in a Tris buffer solution; MV (□), MV8 (○), MV48 (●) and MVG (■) microcapsules. The release profiles from MV48 at constant temperatures, 40 (△) and 25 °C (▲), are also shown in the figure. Two sets of experiments were averaged where the deviation was within 6%.

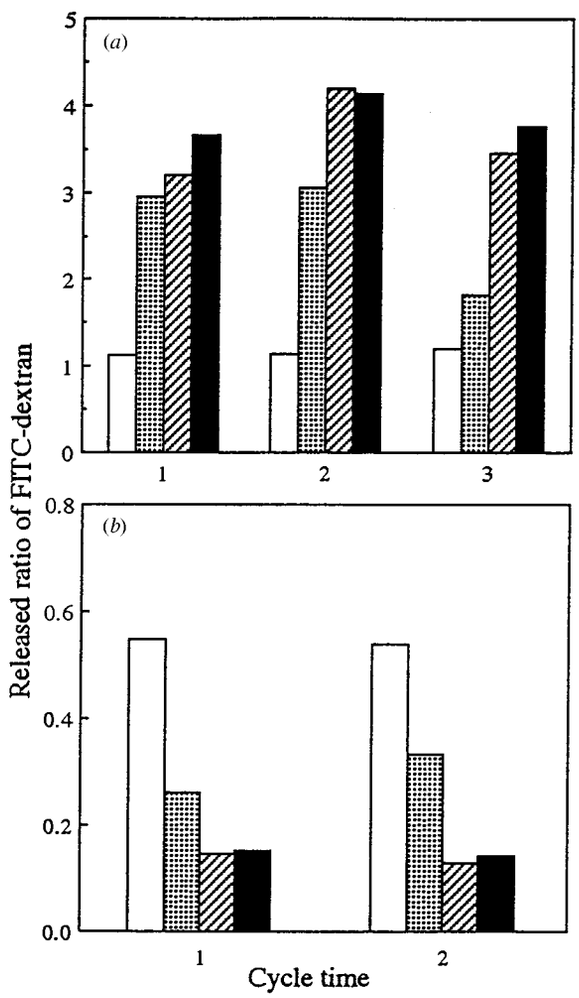


Fig. 6 The enhancement (*a*) and suppression (*b*) of the release rate of MV (unfilled), MV8 (dotted), MV48 (striped) and MVG (filled) microcapsules upon temperature change from 25 to 40 °C (*a*) and from 40 to 25 °C (*b*), respectively. The microcapsules were kept at 25 or 40 °C over a long period (12 h). Two sets of experiments were averaged where the deviation was within 8%.

amounts of FITC-dextran released from microcapsules of the MV series were less than those from microcapsules of the MM series. Since the amount of PNIPAAm gel loaded in the microcapsules of the MV series was smaller than that of the microcapsules of the MM series, the release from the former microcapsules should be more difficult because FITC-dextran should permeate more PLLA membranes encapsulating internal cavities.

The release of FITC-dextran from the microcapsules was investigated over a long period with temperature changes at every 12 h [Figs. 6(*a*) and (*b*)]. Compared with the release from the microcapsules which do not contain PNIPAAm gel, the release from the microcapsules containing PNIPAAm gel is particularly fast at 40 °C, but slows down at 25 °C. The release rate at 40 °C is almost four times that at 25 °C. It is clearly shown in Fig. 6 that the enhanced release at 40 °C is due to gel contraction and not to higher diffusion rate at the high temperature. The latter factor causes only about a 1.2-fold increase in the release rate. Similarly, the reduced release rate upon temperature change from 40 °C to 25 °C is due to gel swelling.

The pattern of temperature-dependent release was reproducible in the experiment involving repeated temperature change, although the amount of dextran released decreased gradually with the repeat time due to the decrease in the dextran left in the microcapsules. The amount of FITC-dextran released is not necessarily proportional to the duration of maintaining the microcapsule at 40 °C. As explained above, the FITC-dextran is squeezed from the gel at a high rate soon after raising the temperature. With the lapse of time, formation of a dense skin layer around the gel may suppress further release of FITC-dextran.

The microcapsules studied here showed a positive thermoresponse. These types of microcapsules may be applied to the release of pesticides and other drugs which should be dosed at high temperature. The threshold temperature can be changed in the range of 20–40 °C by using copolymer gel composed of NIPAAm and hydrophobic or hydrophilic comonomer.[4,16–18]

Conclusions

The PLLA microcapsules holding a number of small poly(NIPAAm) gels inside were prepared and the release of FITC-dextran was studied. The release of FITC-dextran from these microcapsules was thermoresponsive with an increasing amount of dextran released at a high temperature and a decreasing amount at a low temperature. The size and the amount of PNIPAAm gels loaded in the microcapsules were found to be important in controlling the rate and the amount of FITC-dextran release.

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