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Development of pH-Responsive Core-Shell Microcapsule Reactor

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Abstract

A novel type of intelligent microcapsule reactor system was prepared. The reactor can recognize pH change in the medea and control reaction rate by itself. For the reactor system, acrylic acid (AA), *N*-isopropylacrylamide (NIPAM), and glucose oxidase (GOD) were selected as a pH-responsive device, a gating device according and a reaction device, respectively. Poly(NIPAM-co-AA) (P-NIPAM-co-AA) are known to change its hydrophilicity-hydrophobicity due to pH change. They were integrated in a core-shell microcapsule space. GOD was loaded inside the core space and the pores in the outside shell layer were filled with P-NIPAM-co-AA linear grafted chains as pH-responsive gates by plasma graft filling polymerization method. When P-NIPAM-co-AA gates are hydrophilic at high pH value, this microcapsule permits glucose penetration into the core space and GOD reaction proceeds. However, when P-NIPAM-co-AA gates are hydrophobic at low pH value, this microcapsule forbids glucose penetration and GOD reaction will not occur. The accuracy of this concept was examined.

1. Introduction

Our group is investigating functionalized materials, inspired by living bodies. For example, inspired by a K^+ ion channel at biomembranes, Ito *et al.* have developed a molecular recognition ion gating membrane, which can control solvent flux[1] or solute rejection[2] in response to specific ion signals.

Cells can recognize environmental changes at their cytoplasmic membranes and accordingly exchange information at subcellular organelles in cytoplasms. And these cells build up organs which are very macro- control-systems against various stimuli. This is the substancial difference between living organisms and conventional materials, so it is very exiting to develop such a well-control system against environmental changes as cells or organs.

Inspired by such cells; units of the elaborate system, in this study we tried developing a novel type of pH-responsive microcapsule reactor system, which can transfer chemical signals. For this system, methacrylic acid(MAA) and acrylic acid(AA), *N*-isopropylacrylamide(NIPAM), and glucose oxidase(GOD) were selected as a pH-responsive device, a gating device according and a reaction device, respectively. Fig. 1. shows the concept of this study. They were integrated in a core-shell microcapsule space; GOD were loaded inside the core space and the pores in outside shell layer were filled with P-NIPAM-co-MAA or P-NIPAM-co-AA linear grafted chains as pH-responsive gates by plasma graft filling polymerization method[3]. Poly(*N*-isopropylacrylamide)(P-NIPAM) is one of the most popular temperature-responsive polymers since it shows a very sharp phase transition at 32℃. And its significant feature is the ability to design the LCST by incorporating comonomers. MAA and AA are famous vinyl-monomers which have ionizable carboxyl groups sensitive to environmental pH, and the degree of ionization of carboxylic acid is determined by the pH of environment. So the copolymers, Poly(NIPAM-co-MAA)(P-NIPAM-co-MAA) and poly(NIPAM-co-AA)(P-NIPAM-co-AA) are pH-responsive phase transition polymers[4,5].

When this microcapsule reactor is dispersed in the media at high pH value, P-NIPAM-co-MAA or P-NIPMA-co-AA gate polymers turn to be hydrophilic, so this microcapsule reactor permits glucose penetration into the core space and GOD reaction proceeds. However, when this microcapsule reactor is dispersed in the media at low pH value, P-NIPAM-co-MAA or P-NIPAM-co-AA gate polymers turn to be hydrophobic, so this microcapsule reactor forbids glucose penetration and GOD

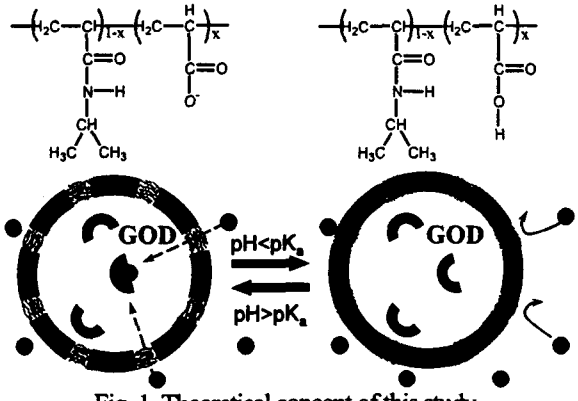

Fig. 1. Theoretical concept of this study

reaction doesn't proceed.

Indeed, GOD loaded microcapsules[6] and pH-responsive microcapsules[7] have been studied by many researchers, but their conventional preparation methods caused disactivation of GOD or unsatisfactory performance of pH-responsibility and endurance. So in this study, "bottle-in method" and "plasma graft filling polymerization method" , which had never been examined,were proposed and investigated to build up this environmental-responsive microcapsule reactor system.

2. Experimental

2.1. Materials

Terephthaloyl dichloride (TDC) was purchased from the Tokyo Kasei Kogyo Co., Ltd., Japan. β-D(+)-glucose was purchased from Sigma Chemical Co., USA). Glucose oxidase from *Aspergilus sp.* was purchased from Toyobo Co., Ltd, Japan. Ethlenediamine (EDA), sodium dodecyl sulfate (SDS), poly(vinyl alcohol) (PVA; average molecular weight 1.23×10^5, 86-90% hydrolysis), benzene, xylene, sodium carbonate, methacrylic acid (MAA) and acrylic acid (AA) were all purchased from Wako Pure Chemical Industries, Ltd., Japan. All the chemicals used were all reagent grade. MAA and AA were used after distillation. The other chemicals were used as received and without any further purification. *N*-isopropylacrylamide (NIPAM) was kindly provided by Kohjin Co., Ltd., Japan, and was used after purifying by recrystallization in hexane and acetone, and then dried in vacuo at room temperature.

2.2. Preparation of Core-Shell Microcapsules by SPG Membrane Emulsification and Interfacial Polymerization

In order to prepare mono-dispersed O/W emulsions containing TDC monomer in SPG membrane emulsification step, an SPG membrane (SPG Technology Co., Ltd., Miyazaki, Japan) and an SPG membrane emulsification kit (Kiyomoto Iron Works Co., Ltd., Miyazaki, Japan), as shown in Fig. 2, were used The SPG membrane was tube-shaped with an outer diameter of 10mm and pore sizes of 11.9 μm. First, 10ml of organic solvent (benzene/xylene=2:1[v/v]) containing 1.5M TDC monomer was pressurized using nitrogen to the continuous phase; 150ml of water containing SDS with concentration of 0.50wt% and PVA with concentration of 0.50wt%.. When the pressure reached 0.01 ~ 0.02MPa, emulsion particles was formed on the surface of the membrane and emulsification began with magnetic stirring.

And in order to prepare mono-dispersed core-shell microcapsules in interfacial polymerization step, to the emulsions 20ml of water containing 1.18M sodium carbonate and 32ml of EDA monomer were added. Polymerization time was 5 min. Then these microcapsules were separated by centrifiguation, and washed three times to remove any emulsifier and unreacted monomer.

These microcapsules were observed using a field emission scanning electron microscope (FE-SEM; S-900, Hitachi, Japan), and analyzed using micromeritics (ASAP-2010 Shimadzu Co., Japan).

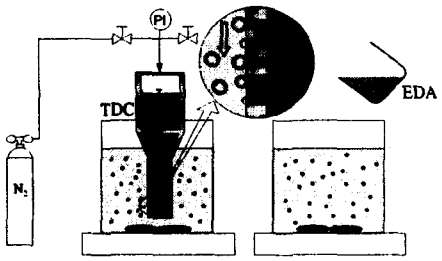

Fig.2 Schematic illustration of microcapsule preparation

2.3. Loading GOD into Microcapsules; "Bottle-In Method"

4.0wt% GOD solution was prepared with acetate buffer(pH=4.6). Microcapsules were immersed into the GOD solution after the solution was once degassed. In general, enzyme-loaded microcapsules are prepared from W/O emulsions which contain enzymes in the core and interfacial polymerization method is employed to form core-shell microcapsules. So, some experimental condition results in deactivation of enzymes[8]. But "bottle-in method" has never been examined.

2.4 Selection of pH-Responsive Gate Polymers and Its Performance

In order to confirm the possibility of grafting NIPAM-MAA and NIPAM-AA onto the organic materials, plasma-graft pore-filling polymerization was employed according to the method described previously[3]. 5wt% of NIPAM-MAA and NIPAM-AA solution was prepared using deionized water and acetate buffer (pH=4.6).For the easy confirmation by Fourier transform infrared spectroscopy (FT-IR; Magna IR 560 with Nic-Plan, USA), porous polyethylene film (supplied by Asahi Chemical Co., Ltd., Japan) was used as the organic materials.

And then, in order to check the performance of the pH-gate P-NIPAM-co-AA polymer, P-NIPAM-co-AA-grafted microcapsules were dialyzed against aqueous glucose with a known concentration. The concentration of glucose in the media was measured by the glucose concentration analyzer (GLU-12, TOA Electronics Ltd., Japan), and the permeability coefficient, *P* could be calculated using the following equation derived from Fick's first law of diffusion[9]:

$$P = \frac{V_s V_m}{A(V_s + V_m)t} \ln\left(\frac{C_f - C_i}{C_f - C_t}\right)$$

where C_i, C_t, C_f are the initial, intermediary (at time t), and final concentrations of glucose in the surrounding medium, respectively. The parameter V_m and A are the total volume and the total surface area of microcapsules, respectively. V_s is the volume of the surrounding medium. This experiment was carried out at pH=4.0 and pH=5.0, and 40℃.

2.5. Effects of plasma graft filling polymerization on GOD

In order to examine the influence of the plasma graft filling polymerization condition on GOD, the activities of (1)GOD treated with Ar plasma (30W, 60sec) and (2)GOD treated with Ar plasma (30W, 60sec) and immersed in NIPAM-AA monomer solution (1day) were measured with time due to the concentration of dissolved oxygen(DO). Concentration of DO was measured using DO-24P(TOA Electronics Ltd., Japan).

2.6. Demonstration of pH-Responsive Microcapsule Reactor

Onto the pores in the shell layer of GOD-loaded core-shell microcapsules P-NIPAM-co-AA was grafted. Then the apparent activities of these microcapsules were measured at pH=4.0 and pH=5.0 at 40℃. It was calculated from the concentration of DO.

3. Results and discussions

3.1. Preparation of Core-Shell Microcapsules by SPG Membrane Emulsification and Interfacial Polymerization

Fig. 3. shows the FE-SEM micrographs of the entire figure, cross-sectional view, outer and inner surface of the microcapsules. By using SPG membrane emulsification method, emulsions with the size of 3.5 times as large as the pore diameters are prepared; at this study, 11.9 μm×3.5= 41.65 μm. As seen in Fig. 3., the diameters of the microcapsules are around 42 μm. So this two step method is suitable for preparing size-controlled microcapsules. Structural parameters of the microcapsules elucidated by FE-SEM and BET measurements are shown in Table 1.

3.2. Loading GOD into Microcapsules; "Bottle-In Method"

Kamyshny *et al.* have reported that DLS revealed the size of GOD is 7±1nm[10]. And as shown in Table 1, the pore diameter of the microcapsules was 16nm and the thickness of the shell of the microcapsules was 1 μm, so we thought GOD probably passes through the shell membrane. This predict was supported by 'pore model' proposed by Nakao *et al.*[11]. This model showed us that diffusion coefficient of GOD in the shell membrane became 0.32 times as large as one in the dilute solution and about 5 minutes is enough to load GOD into the microcapsules using dialysis.

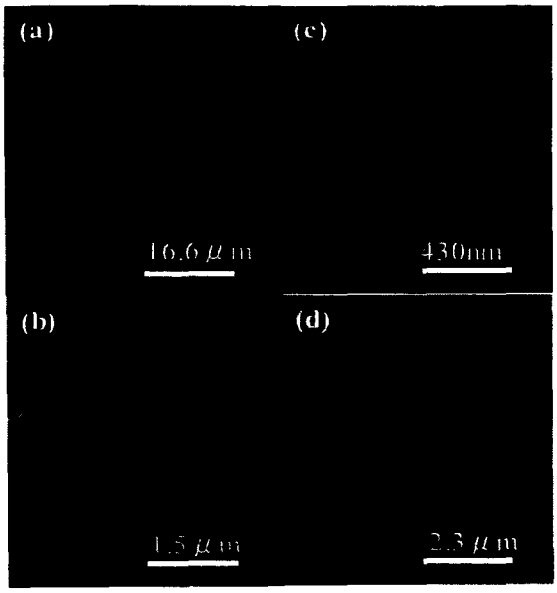

Fig.3 FE-SEM micrographs of the microcapsules; (a)entire figure, (b)cross-section, (c)outer surface, (d)inner surface

Fig. 4. shows the relationship between inclusion time and inclusion amount. This "bottle-in method" need only about 30minitues to load GOD.

In this study, one unit causes the formation of one micromole of hydrogen peroxide per minute at pH=4.6 at 40℃.

3.3. Selection of pH-Responsive Gate Polymers and Its Performance

P-NIPAM-co-AA was successfully grafted, but P-NIPAM-co-MAA was not grafted.

Indeed peroxide method[2] or Ce^{4+} initiated grafting method[7] could be employed to prepare pH-responsive gates, but both methods results in deactivation of enzymes. So plasma graft filling polymerization method and P-NIPMA-co-AA were selected to form pH-responsive gates, and the gates polymer, respectively.

Fig. 5. shows the effect of pH on the permeability of glucose from P-NIPAM-co-AA grafted microcapsules. Grafting time was 1 day. At pH=5.0 the permeability of glucose was 2.6 times as large as that at pH=4.0. This difference resulted from hydrophobicity-hydrophilicity change between pH=4.0 and pH=5.0.

3.4. Effects of plasma graft filling polymerization on GOD

Fig. 6. shows the effect of the operations during plasma graft filling polymerization on the activities of GOD. The activities

Table 1 Structural parameters of the microcapsules

Diameter [μm]	42
Thickness of the shell [μm]	1
BET surface area [m^2/g]	13
Average pore diameter [nm]	16

shown are relative to those of untreated GOD. In this study, activities were defined at pH=4.6, 40℃. Neither (1)Ar plasma treatment(30W, 60sec) nor (2)monomer solution supply(1day) caused deactivation of GOD. This means that plasma graft filling polymerization method is very useful to endow the gating function to enzyme-loaded microcapsules.

3.5. Demonstration of pH-Responsive Microcapsule Reactor

Fig. 7. shows the apparent activity at pH=5.0 relative to that at pH=4.0. The temperature was 40℃. The relative apparent activity at pH=5.0 was 2.7 times larger. This result agrees well with the result of the permeation test of glucose. It showed that at pH=5.0 the permeability of glucose was 2.6 times as large as that at pH=4.0. These results suggest as follows. When the pH in the media is 5.0, P-NIPAM-co-AA gate polymers was hydrophilic and so glucose could penetrate through the shell layer and GOD reaction proceeded. However, when the pH in the media is 4.0, P-NIPMA-co-AA gate polymers turned to be hydrophobic and so glucose could not penetrate through the shell layer and GOD reaction didn't proceed. That is, in this system the diffusion of glucose is rate-limiting step. So the difference of the apparent activity could be beared.

4. Conclusions

In this study, the core-shell microcapsules were prepared by two steps; SPG membrane emulsification and interfacial polymerization. The pores of these microcapsules are relatively large, so we demonstrated the efficiency of " bottle-in method", which had never been studied. By this method, enzymes can be loaded in a short time.

Second, the microcapsules, which is composed of core-shell porous membrane and linear grafted P-NIPAM-co-AA chains in the pores acting pH gates, could control the permeability of glucose, depending on the pH in the media.

Third, we showed that plasma graft filling polymerization method was very useful to graft functional polymer, maintaining activities of GOD.

And finally, we demonstrated the concept of this study; pH-responsive core-shell microcapsule reactor. system In developing this system, it was very important to design the diffusion-limiting-step of the substrate; glucose.

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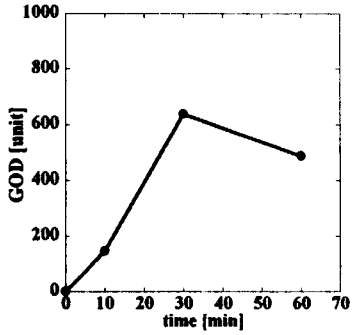

Fig. 4 GOD loading by "bottle-in method"

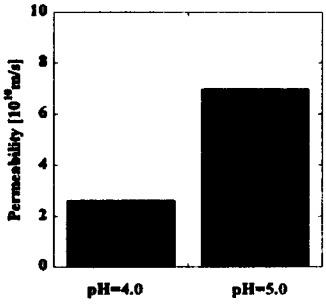

Fig.5 Permeability of glucose (40℃)

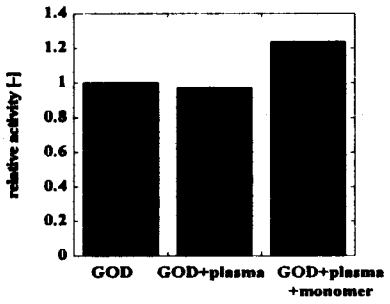

Fig. 6 Effects of plasma graft filling polymerization method

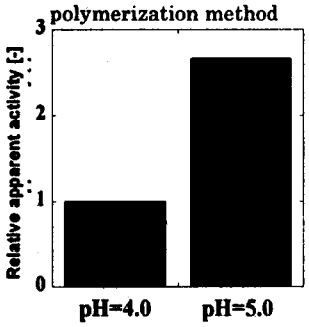

Fig.7 Relative apparent activity of the microcapsules (40℃)

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