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Preparation of W/O/W microcapsule containing enzyme without alcohol

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ABSTRACT

We proposed a novel method for the preparation of W/O/W type microcapsules containing enzymes. In this method, a gas phase was used in place of alcohol that has been conventionally used in solvent change processes for the preparation of W/O/W type microcapsules. As a model system for this study, we prepared glucose-oxidase encapsulated poly(L-lysine-alt-terephthalic acid) microcapsules whose volume can be decreased in the presence of glucose and evaluate the effect of glucose on the shrinking process. To estimate the relative concentration of enzyme in microcapsule, a simple model was proposed on assumption of linear dependence of the rate of change in the size and in the charge density of the microcapsule membrane. The diameters of the shrunk microcapsule observed were consistent with the theoretical value. By a fitting of the observed time course of the microcapsule size to the theoretical equations, two time constants were obtained, both related inversely to the relative enzyme concentration in the microcapsules. From the comparison of their constants for microcapsules prepared by the novel and conventional method, it became apparent that the glucose-oxidase containing microcapsule prepared by the novel method exhibited about 1.5 times higher enzyme activity of the case for the conventional method using ethanol in the solvent change processes.

1. Introduction

Immobilization of enzyme into microcapsules is of interest for application to wastewater treatment because it allows us not only degradation but also recuperation of valuable components from effluent streams [1–3]. To maintain the enzyme activity in the microcapsules, we should not use alcohol for the preparation of the microcapsule because of enzyme denaturation, though alcohol is usually used in preparation of water-in-oil-in-water (W/O/W) type microcapsule [4–9]. In this study, we proposed a novel method for preparing the enzyme-containing W/O/W microcapsule with a high catalytic ability. This method used a gas phase in place of alcohol to exchange oil for water when O/W type microcapsule was converted into W/O/W type one. To assess the efficacy of the method, we prepared a glucose-sensitive microcapsule whose volume changed by glucose oxidation by encapsuled a glucose-oxidase into a pH sensitive W/O/W microcapsule, and also evaluated the apparent activity of glucose oxidation from the volume change of the glucose-sensitive microcapsule. The microcapsule was prepared by including glucose-oxidase into poly(L-lysine-alt-terephthalic acid) (abbreviated hereafter to PPL) microcapsules, as pH-sensitive microcapsule [6–9]. The enzyme activity of the glucose-oxidase containing PPL microcapsule prepared with alcohol and without alcohol was evaluated from the volume change of the PPL microcapsule whose volume changed with the glucose oxidization of inner microcapsule. The enzyme activities were examined as a function of the ethanol immersion period in the microcapsule preparation. A proportional decline of the activity was observed with the ethanol immersion period.

2. Theoretical background

Fig. 1 illustrates the concept of volume change of glucose-sensitive microcapsule (glucose-oxidase containing PPL microcapsule). PPL microcapsules swell up to about pH 6, while shrink down to it [6,7]. Glucose oxidization by glucose-oxidase products gluconic acid and hydrogen peroxide [10,11], resulting in decline of external pH. In a glucose water solution, the swelling glucose-oxidase containing PPL microcapsule, hence, can shrink through the pH decreasing by the glucose oxidization.

The theory of the shrinking process of PPL microcapsule can be based on the idea that the shrinking results from competition between the elasticity and the charge repulsion of the microcapsule membrane [8,9]. The elastic property of microcapsule membrane is generally described in terms of its size. The electric property of microcapsule membrane is described by an introduction of "effective charge". Then, the thermodynamic state of a microcapsule is assumed to be expressed by the two parameters; the "effective" charge of the membrane q and the diameter D of the microcapsule. It should be noted that the diameter of microcapsule is observed

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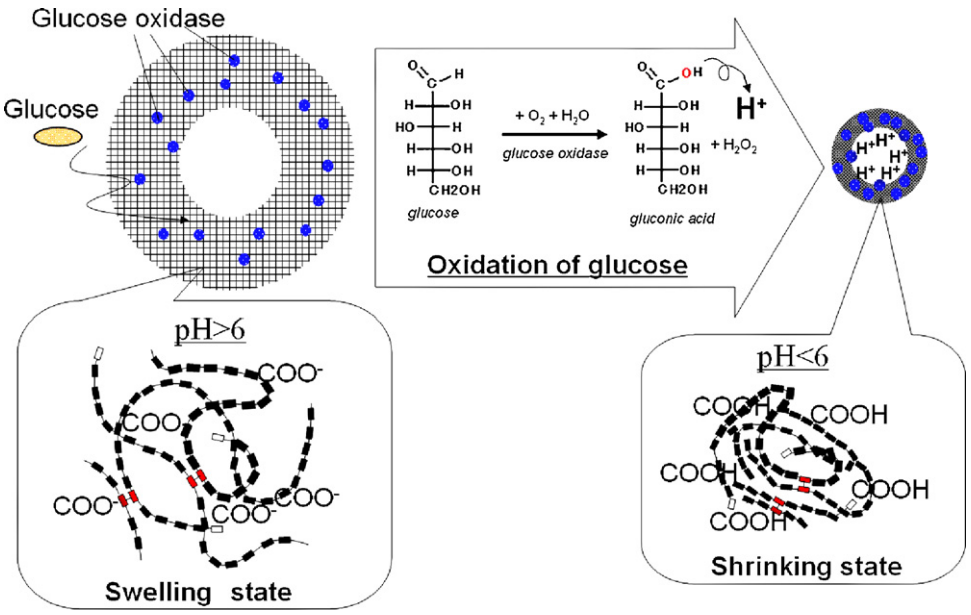

Fig. 1. Schematic diagrams of glucose-sensitive microcapsule; PPL microcapsule membrane is shrank by dissociated proton from gluconete that produced from oxidization of glucose.

directly but the effective charge is not. Therefore, to compare the theoretical result with the experimental one, the final theoretical result must be expressed in terms of only the diameter D. On the basis of the conventional non-equilibrium thermodynamics, the relaxation process is expressed by the time development of the thermodynamic quantities of D.

Let us consider the time development from the initial state (q_i, D_i) to the final equilibrium state (q_{eq}, D_{eq}). It is reasonable to assume that the driving force F is proportional to the "quench depth" $D(t) - D_{eq}$. Thus,

$$F = -k_1(D(t) - D_{eq}) \tag{1}$$

The membrane shrinking is induced by diminution in the repulsion, which is originated from negative charges of the membrane. When the driving force for decreasing D in the shrinking linearly relate to effective charge $q(t)$, the constant k_1 should depend on $(q_i - q(t))$ and vanish at $q = 0$. When the charge reducing rate α was higher than the deformation speed of membrane, the flow velocity of materials through the membrane is higher than that in the apparent diminution. The charge relaxation time, which relate inversely with the charge reducing rate α, is known to be shorter than the elastic relaxation time, which relate inversely with the deformation speed of membrane, in PPL membrane from previous studies [8,9]. Thus, the constant k_1 can be simply expressed by $k_1 = k_0\alpha(q(t)-q_i)$, where k_0 is a positive constant. Consequently, the time development equation of the diameter is given by

$$\frac{dD(t)}{dt} = K_0\alpha(q(t) - q_i)(D_{eq} - D(t)) \tag{2}$$

The charge of PPL microcapsule membrane $q(t)$ simply reach a maximum at about pH 7 and a minimum at about pH 4.5 [6–9] under the equilibrium situation. In the pH 4.5 to 7 region the membrane charge $q(t)$ are can be expressed by the reducing rate of charge α. The initial and equilibrium charge can be also described as q_i and 0, respectively;

$$\begin{aligned} q(t) &= q_i && \text{at pH} \geq 7 \\ q(t) &= q_i - \alpha t && \text{at } 4.5 < \text{pH} < 7 \\ q(t) &= 0 && \text{at pH} \leq 4.5 \end{aligned} \tag{3}$$

The initial state and the equilibrium state are denoted by (q_0, D_0) and (q_{eq}, D_{eq}), respectively.

Above pH 7, Eq. (2) can be solved as

$$D(t) = D_0 \quad \text{at pH} \geq 7 \tag{4}$$

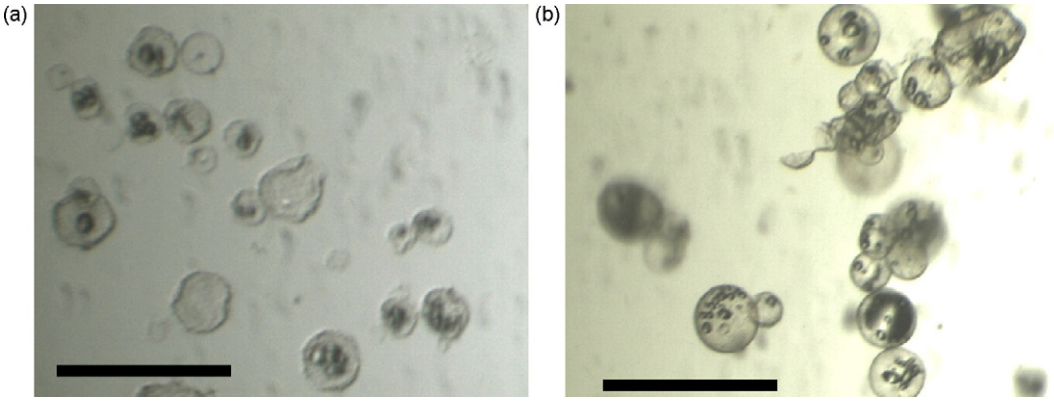

Fig. 2. Microphotographs of the glucose-oxidase-including PPL microcapsule in water at pH8 prepared (a) with ethanol and (b) without ethanol. The lengths of lines in the pictures are 1 mm.