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Original Research Paper

Preparation of calcium carbonate microparticles containing organic fluorescent molecules from vaterite

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ABSTRACT

The encapsulation of fluorescent organic molecules into crystalline calcium carbonate was examined using calcium carbonate microcapsule, whose crystalline phase is vaterite as a metastable phase of calcium carbonate. A calcium carbonate microcapsule with impregnated pyrene that is a water insoluble fluorescent molecule was soaked into suitable aqueous solutions to promote the phase transition of vaterite toward calcite as the stable phase of calcium carbonate. When 0.2 M calcium chloride solution was used, the largest amount of pyrene (approximately 0.06 wt%) was encapsulated into the calcite particle. Pyrene thus included was not eliminated even after thorough washing with THF. The calcite particle thus prepared produced the excimer emission of pyrene by UV irradiation. Rhodamine B was also introduced into calcium carbonate by the immersion of the microcapsule into the aqueous solutions of Rhodamine B. The fluorescence of rhodamine B was observed from the calcium carbonate particles by visible light irradiation. Acetaminophen, a common drug poorly soluble in water, was also included in the calcium carbonate particle by the same procedures as the pyrene encapsulation. As acetaminophen thus encapsulated was released by the dissolution of the calcium carbonate particle in acidic solution, the particle is expected to be applied for a dissolution-triggered drug delivery.

1. Introduction

Fluorescent molecules and materials are useful probes for the extensive analyses of genes, proteins, drug targets in cells, tissues and other living systems [1–6]. While organic dyes and fluorescent proteins are still convenient materials [1–3], inorganic quantum dot microparticles are also applied to the biological sensing researches [4–6]. Since the direct use of these fluorophores and fluorescent materials often encounter some severe problems such as pH, pro-oxidant species and dissolved salts within organism environments, their functionalizations and decorations are usually required. However, the elimination and the egestion of these artificial decorations into living organisms must be carefully controlled. Calcium carbonate is one of the most useful and versatile materials for biomedical fields [7–10]. Its high environmentally-friendly property and biocompatibility are undoubtedly appropriate for a broad range of utilizations for living objects and human being. As calcium carbonate is readily decomposed or metabolized within organisms to non-toxic calcium and carbonate ions (or carbon dioxide), calcium carbonate materials with properly designed functions must be powerful tools for biomedical related systems, including bio-sensing and drug delivery [11–17].

The preparation of fluorescent calcium carbonate materials have been examined by the doping of fluorescent metal cations such as Gd, Eu [18,19], Sn [20] and Ce [21], because the ion-exchange of calcium to these metals effectively occurs due to their analogous ionic radii. Recently, the precipitation of calcium carbonate in the presence of mercaptopropyl acid-modified CdTe is reported to be an efficient method for incorporating quantum dot microparticles into crystalline calcium carbonate, which is beneficial for monitoring intracellular uptake [22]. On the other hand, as a number of fluorescent organic molecules and fluorescent pigments are known [23,24], the incorporation of these common phosphors in calcium carbonate must be versatile approaches to the preparation of fluorescent calcium carbonate materials. Although amorphous calcium carbonate [25–30] is used as a template for the encapsulation of fluorescent molecules into organic capsules like chitosan [31] and PMMA [32], the crystalline calcium carbonate materials containing fluorescent organic

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molecules have not been examined. Therefore, new methods for the encapsulation of fluorescent organic molecules into crystalline calcium carbonate are required.

We have studied the preparation of inorganic hollow microparticles (microcapsules) [33–35] such as calcium carbonate [36–39] by an interfacial reaction method. The crystalline phase of the calcium carbonate microcapsule is vaterite as a metastable phase of calcium carbonate, while the common calcium carbonate observed in nature is calcite as the most stable phase. We have recently found that the phase transition of the vaterite calcium carbonate microcapsule to calcite in the aqueous solutions of proteins such as bovine serum albumin (BSA), lysozyme and insulin achieves the encapsulation of those proteins into the resulting calcite calcium carbonate [40]. The application of this phase transition method to the incorporation of organic molecules is expected to develop a new procedure for creating functional crystalline calcium carbonate materials with organic molecules. In this paper, we report the encapsulations of pyrene and rhodamine B as fluorescent molecules into crystalline calcium carbonate particles by the phase transition method. Moreover, the incorporation of acetaminophen, a poorly water-soluble organic molecule, into calcium carbonate particle and its release behavior are also described.

2. Materials and methods

2.1. Materials

Ammonium carbonate [$(NH_4)_2CO_3$], calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), pyrene, rhodamine B and other solvents (n-hexane, THF, methanol and ethanol) used in this work were purchased from Wako pure chemical industries. Tween 85 and a commercial calcite calcium carbonate were obtained from Kanto Chemicals and acetaminophen was from MP biomedicals. 1 M Tris HCl solution (pH = 7) was commercial available from Wako Pure Chemical Industries and 0.2 M calcium chloride solution was prepared with calcium chloride dihydrate in our laboratory. The structures of molecules encapsulated into calcium carbonate are summarized in Fig. 1.

2.2. Preparation of vaterite calcium carbonate microcapsule

Calcium carbonate microcapsules were prepared by a described method in our recent papers [39,40]. A typical procedure is briefly describe as follows: 32 mL of a 3 M ammonium carbonate solution was mixed with 48 mL of a n-hexane solution dissolving 1.0 g of Tween 85, and the resulting solution was emulsified with a homogenizer at 8000 rpm for 1 min in order to obtain a water/oil (W/O) emulsion. This W/O emulsion was poured into 640 mL of a 0.3 M calcium chloride aqueous solution in one portion at 30 °C. After 5 min stirring, the precipitate was filtered, washed with deionized water twice and methanol once and finally dried at 80 °C for 12 h.

2.3. Encapsulation of pyrene and acetaminophen into calcium carbonate particles

This procedure consisted of two processes; the first step is the impregnation of pyrene onto the calcium carbonate microcapsule and the second one is the immersion of the microcapsule into an aqueous solution. The calcium carbonate microcapsule (1 g) was soaked in 50 mL of a THF solution with dissolved pyrene (0.2 g). After gentle mixing of this suspension for 30 min, the volatiles were removed under reduced pressure. This calcium carbonate microcapsule with impregnated pyrene was added to 20 mL of a 1 M Tris HCl buffer solution or a 0.2 M calcium chloride solution. The resulting suspension was stood at 22 °C for 24 days. The aqueous solution and white skim on the solution (identified as pyrene) were carefully separated from the calcium carbonate solid by decantation. The calcium carbonate solid was washed with deionized water more than three times, and finally dried at 60 °C for 12 h. This calcium carbonate was further washed with THF in order to remove pyrene until pyrene was not detected in the THF solutions by UV–vis spectrometry analysis (Jasco V-530 spectrophotometer). Double washing was enough to remove pyrene from the calcium carbonate particle completely. The encapsulation of acetaminophen was carried out by the same procedures as pyrene.

2.4. Encapsulation of rhodamine B into calcium carbonate particles

The calcium carbonate microcapsule (1 g) was immersed in 10 mL of a 1 M Tris HCl buffer solution or a 0.2 M calcium chloride solution with 0.2 g of rhodamine B. The resulting suspension was stood at 22 °C for 24 days. This solid was filtered, thoroughly washed with deionized water, and dried at 60 °C for 12 h. This solid was further washed with ethanol thoroughly until rhodamine B was not detected in the solutions by UV–vis spectrometry analysis. More than three times washing was necessary to remove the rhodamine B from the calcium carbonate particle perfectly.

2.5. Determination of the weight ratios of organic molecules encapsulated into calcium carbonate particles

The amounts of organic molecules encapsulated into the calcium carbonate particles were estimated by the extraction of the molecules. The calcium carbonate particles were dissolved with an 8.5 M acetic acid solution, and the amounts of eliminated molecules were determined by UV–vis spectrometry (Jasco V-530 spectrophotometer). In the cases of acetaminophen and rhodamine B, after the complete dissolution of the calcium carbonate particles, the resulting acetic acid solutions were directly analyzed by the spectrometer. In the case of pyrene, after the complete dissolution of the calcium carbonate particles, pyrene was extracted from the solutions with toluene, and the pyrene contents in the toluene solutions were analyzed by the spectrometer. The extracted contents of pyrene, rhodamine B and acetaminophen were determined

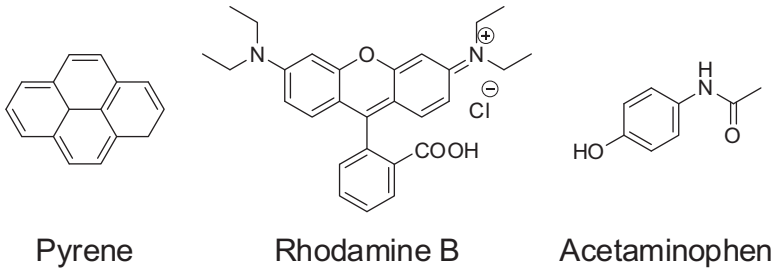

Fig. 1. Structures of molecules encapsulated into calcium carbonate.