Fabrication of Yttrium Phosphate Microcapsules by Emulsion Route for In Situ Cancer Radiotherapy

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Abstract

Radiotherapy is a novel, non-invasive cancer treatment. Radioactive hollow microspheres, i.e., microcapsules, are attractive for in situ cancer radiotherapy because they can effectively reach tumors without settling in blood vessels. In particular, microcapsules 20-30 µm in size are expected to exhibit not only a radiotherapy effect but also embolization that blocks the nutrient supply to cancer cells. β-ray irradiation is the most suitable source for in situ radiotherapy because of its moderate range. Several kinds of β-emitting yttria (Y₂O₃) microcapsules have therefore been developed. Yttrium phosphate (YPO₄) should have a longer irradiation effect than that of Y₂O₃ because the half-life of ³²P (14.3 days) is longer than that of ³⁵S (64.1 hours). However, the preparation of YPO₄ microcapsules has not been reported to date. In the present study, YPO₄ microcapsules were fabricated using a water/oil (W/O) emulsion prepared by first dispersing a YPO₄ sol into toluene containing a surfactant, with mechanical homogenization. The emulsion was then added into butanol to dehydrate the water phase and precipitate microcapsules. These were then heat-treated to improve their mechanical strength and chemical stability. Microcapsule fragility at low YPO₄ sol concentrations in the water phase was attributed to the thinness of the microcapsule shell. The size of the microcapsules decreased with increasing emulsification speed. The chemical stability of the prepared microcapsules is similar to those of previously reported YPO₄ and Y₂O₃ microspheres in weakly acidic conditions. Thus, little leakage of radioactive species into nearby healthy tissues is expected. The obtained microcapsules are expected to be highly effective for cancer radiotherapy.

Keywords: Yttrium phosphate, Microcapsule, Cancer treatment, Radiotherapy, Emulsion, Chemical durability

1. Introduction

Cancer has recently become the main cause of death in Japan [1]. Therefore, the development of efficient cancer treatments is urgently needed. Surgical excision is widely chosen as a treatment course. However, some organs do not recover their functions after surgery. The development of a non-invasive cancer treatment is needed to improve quality of life.

Radiotherapy is a non-invasive cancer treatment but it is difficult to treat deep-seated cancer with low side effects by irradiation from outside the body. Recently, novel radiotherapies using ceramic microspheres imparting β-ray irradiation have been developed [2]. As an irradiation source, a γ-emitter is liable to penetrate into the surrounding healthy tissues and consequently induce side effects, while an α-emitter can convert another surrounding element into a γ-emitter. Therefore, a β-emitter, which has an average range of 2.5 mm, is suitable for in situ radiotherapy. Implanted microspheres reach tumors through blood vessels and can thus locally irradiate a deep-seated tumor. Hollow microspheres (microcapsules) are expected to effectively reach tumors without settling in blood vessels, unlike dense microspheres. In particular, those with a size of about 20-30 µm can shut off the supply of nutrients to cancer cells by embolization [2]. In addition, they are attractive as carriers of anti-cancer agents in drug delivery.

It is known that non-radioactive ⁸⁹Y, with a natural abundance of 100%, can be converted into β-emitting ⁹⁰Y, whose half-life is 64.1 hours, by neutron bombardment [3]. Based on this, microspheres composed of Y₂O₃-Al₂O₃-SiO₂ glass [4-6], Y₂O₃ ceramics [7-10], and ⁹⁰Y-containing resin [11] have been proposed. However, the half-life of ⁹⁰Y is somewhat brief for effective radiotherapy. Non-radioactive ⁹⁰Y, with a natural abundance of 100%, can be converted into β-emitting ⁳⁵P, whose half-life is 14.3 days, by neutron bombardment. Therefore, yttrium phosphate (YPO₄) is expected to possess a longer irradiation effect in vivo than that of Y₂O₃. Although the preparation of dense YPO₄ microspheres
has previously been reported [7,12], that of YPO₄ microcapsules has not.

In the present study, YPO₄ microcapsules were prepared via the precipitation of YPO₄ from a water/oil (W/O) emulsion and subsequent heat treatment. To evaluate the optimal conditions for the preparation of YPO₄ microcapsules suitable for radiotherapy, the effects of YPO₄ concentration in the water phase and emulsification speed on the shape and diameter of the particles were investigated. In addition, the chemical stability of the microcapsules was evaluated in a simulated body environment.

2. Materials and methods

NaOH and 1-butanol were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). 80 mL of 0.56 M Y(CH₃COO)₃ and 20 mL of 2.3 M KH₂PO₄ aqueous solutions were mixed. The resulting precipitates were collected by a centrifugal separator (CN-2060, As-one Co., Osaka, Japan) and washed with ultrapure water. They were then dispersed in 15 or 30 mL of 0.1 M HNO₃ and stirred at room temperature for 24 h to obtain a YPO₄ sol. 5 mL of the sol were then mixed with 50 mL of toluene containing nonionic surfactant (Span80) at 0.5 mass% and emulsified at various speeds, ranging from 2000 to 3000 rpm, for 10 min using a rotary homogenizer (Homo Mixer Mark II, Tokushu Kika Co., Osaka, Japan). The resultant W/O emulsion was immediately added to 150 mL of 1-butanol and stirred for 1 min. The precipitates were filtered and dried at 60 °C for 24 h, heated to 1000 °C at a rate of 5 °C/min, and kept at that temperature for 2 h.

The microstructure of the powder product was characterized by X-ray diffraction (XRD; MXP3V, Mac Science Ltd., Japan), scanning electron microscopy (SEM; S-3500N, Hitachi Co., Japan), and an energy-dispersive X-ray analyser (EDX; Model EX-400; Horiba Co., Kyoto, Japan). The particle size distribution was characterized by measuring the size of each particle from SEM images. The chemical stability of the microcapsules was examined as follows. 50 mg of microcapsules were kept in 20 mL of 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer for 10 days at 36 °C under vigorous stirring. The pH of the MES solution was buffered at 6 by the dropwise addition of 1 M NaOH because the pH around tumors is reported to be sometimes weakly acidic through the secretion of lactic acid [13]. Y and P concentrations released from the microcapsules into the buffer were measured by inductively coupled plasma (Optima 4300DV Cycleron, Perkin-Elmer Co., England) atomic emission spectroscopy (ICP-AES). The fractions of Y and P released from the microcapsules were calculated using the following equation:

\[
\text{Released fraction} = \frac{\text{Molar quantity of Y or P released from the sample into MES (mol)}}{\text{Total molar quantity of Y or P contained in the sample (mol)}}
\]

3. Results

Figure 1 shows SEM images of the non-heated particles prepared from YPO₄ sol mixed with either 15 or 30 mL of HNO₃ solution. Microspheres with good spherical shapes were obtained in the former, while almost all of the particles were broken in the latter. A YPO₄ sol containing 15 mL of HNO₃ solution was used for subsequent sample preparation. Figure 2 shows SEM images and the size distribution of the particles obtained at various emulsification speeds after heat treatment at 1000 °C. The particle sizes ranged from 5 to 50 µm, and tended to decrease with increasing emulsification speed. Figure 3 shows a SEM image of cross-sections of the particles after heat treatment at 1000 °C. The obtained microspheres were found to have a hollow structure. Figure 4 shows XRD patterns of the microcapsules after heat treatment at 1000 °C. The sample was amorphous before heat treatment (Data not shown), while peaks assigned to crystalline tetragonal xenotime-type YPO₄ (JCPDS card no. 11-0254) were observed after heat treatment. The crystallinity of the samples increased with increasing heat treatment temperature (Data not shown). Fractions of Y and P released from the microcapsules into MES buffer during stability tests were 1.4 × 10⁻² and 6.6 × 10⁻³, respectively. Y/P molar ratios of the microcapsules before and after soaking in MES buffer were 1.27 ± 0.07 and 1.54 ± 0.18, respectively (n = 5).

![SEM images of non-heated particles prepared from a YPO₄ sol mixed with 15 or 30 mL of HNO₃ solution (emulsification speed of 3000 rpm).](image1)

![SEM images of particles fabricated at various emulsification speeds after heat treatment at 1000 °C.](image2)
found that acid-soluble impurities accumulated at the grain boundary of the microspheres, causing low chemical stability [7]. The present results support the view that normal heat treatment is more suitable than quenching for the fabrication of microspheres with high chemical stability.

The released fraction of P was 47 times higher than that of Y, as shown in Table 1. This means that the Y/P molar ratio of the released chemical species differed from that of the original YPO₄ microspheres, which had a molar ratio of about one [7,12]. It has been reported that AlPO₄·1.5H₂O dissolves unevenly, releasing a larger amount of P than Al in weakly acidic conditions of around pH 6, because the released Al³⁺ is hydrated and re-precipitated in this pH region [17]. A similar phenomenon may occur in the case of the present YPO₄ microcapsules. The increase in the Y/P molar ratio of the microcapsules after soaking in MES buffer supports this assumption (see Table 2).

Table 1. Fractions of Y and P released from samples into MES buffer at pH 6 at 36 °C for 10 days (emulsification speed of 2000 rpm).

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<tr>
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<th>Y</th>
<th>P</th>
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<tr>
<td>Before</td>
<td>1.4 × 10⁻⁴</td>
<td>6.6 × 10⁻⁴</td>
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<tr>
<td>After</td>
<td>1.27 ± 0.07</td>
<td>1.54 ± 0.18</td>
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Table 2. Y/P molar ratios of microcapsules before and after soaking in MES buffer at pH 6 at 36 °C for 10 days (emulsification speed of 2000 rpm, n = 5).

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<thead>
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<th></th>
<th>Before</th>
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<tr>
<td>Y/P</td>
<td>1.56 ± 0.06</td>
<td>1.37 ± 0.15</td>
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5. Conclusion

YPO₄ microcapsules with diameters in the range of 20-30 µm were obtained by a wet process in a W/O emulsion. The size of the microcapsules can be controlled well by the emulsification speed. The chemical stability of the microcapsules in buffer solutions was as high as those previously reported for YPO₄ microspheres. These microcapsules are expected to be useful for the in situ radiotherapy treatment of cancer.

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References


