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RESEARCH PAPER

Preparation and Characterization of Chitosan Microspheres Containing Doxifluridine

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ABSTRACT

Chitosan microspheres containing 5-fluorouracil (5-FU), tegafur (FT), and doxifluridine (DFUR) were prepared by the dry-in-oil method using silicone oil with no surfactant as a dispersion medium. For DFUR-containing chitosan microspheres (DFUR-M), reacetylation with acetic anhydride or coating using chitosan and glutaraldehyde was performed. DFUR-M, reacetylated DFUR-M, and chitosan-coated DFUR-M were investigated on in vitro drug release, and the former two microspheres were examined for in vivo degradation after subcutaneous (s.c.) implantation in mice, and in vivo plasma concentration–time profiles after s.c. implantation in rats. The present method gave fairly large microspheres purely composed of chitosan and drug because of no use of surfactant, which showed the mean particle diameters of 300–900 μm and the drug contents of 4–22% (w/w). Encapsulation efficiency of DFUR was higher than that of 5-FU and FT. DFUR-M and reacetylated DFUR-M exhibited spherical shape except chitosan-coated DFUR-M. DFUR-M showed high initial rapid release, which was suppressed to some extent by reacetylation or chitosan coating. DFUR-M and reacetylated DFUR-M subcutaneously implanted were gradually degraded, and approximately half or a little more of the microspheres disappeared from the implanted site at 3 weeks postimplantation. DFUR-M and reacetylated DFUR-M implanted subcutaneously gave similar plasma concentration–time profiles of DFUR, which did not indicate prolonged release in vivo. DFUR-containing chitosan microspheres with fairly large size and good drug content could be obtained by the present preparation but remained to be improved for drug release properties.

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Key Words: Chitosan microsphere; Doxifluridine; Particle size; Drug content; Drug release; Subcutaneous implantation.

INTRODUCTION

Chitin, a poly-β-(1 → 4) linked *N*-acetyl-D-glucosamine, is a polysaccharide widely distributed in nature, and chitosan is obtained by deacetylation of chitin with condensed alkaline solution. Chitin and chitosan are biocompatible and biodegradable polymers,[1–6] and have been developed for biomedical applications including wound dressings[7–9] and drug delivery systems.[10–16] In particular, since chitosan is soluble in aqueous acidic media, it is useful for many pharmaceutical applications. Chitosan is available as a bioadhesive polymer,[17–21] or excipient enhancing drug absorption.[22] The biodegradation characteristics of chitosan are dependent on the degree of deacetylation.[3,4] For example, 50% randomly deacetylated chitosan, soluble in neutral pH, was quickly degraded in the presence of lysozyme.[5] Chitosan microspheres as previously reported, which were prepared using 56% deacetylated chitosan, were degraded gradually in the presence of lysozyme and subcutaneously in vivo.[15] At that time, gradual drug release was also observed in the presence of lysozyme, while in the absence of lysozyme, sufficient drug release was hardly observed. In some cases, drug release from chitosan microspheres is controlled by chemical cross-linking with glutaraldehyde.[23] Thus, chitosan microcapsules or microspheres have been developed as possibly useful drug delivery systems.

The implantable dosage forms are often attempted for tumor treatment. Especially, microparticulate dosage forms have been widely examined for tumor chemotherapy.[12,24,25] They are applicable for chemoembolization, delivery to tumor sites based on cellular phagocytosis, prolonged drug supply around implanted sites, and so forth. The intraperitoneal chemotherapy has been attempted for treatment of peritoneal carcinomatosis or peritoneal recurrence of gastric carcinoma.[26,27] At that time, prolonged release systems such as microspheres of antitumor drugs will reduce the frequency of dosing. Also, regional administration of implanting systems of antitumor agents will allow high drug concentration in the diseased part. Antitumor agents related to 5-fluorouracil (5-FU) exhibit antitumor action in a time-dependent manner.[28] Therefore, the microspheres controlling release of such drugs are suggested as a possibly useful system for tumor chemotherapy. In the present study, preparation of chitosan microspheres containing 5-FU-related antitumor agents [5-FU, tegafur (FT), and doxifluridine (DFUR)] was attempted using the previous dry-in-oil method with no surfactant.[15] The obtained microspheres were characterized on particle size, drug content, and encapsulation efficiency. Further, some types of chitosan microspheres containing DFUR were examined with regard to in vitro drug release and in vivo degradation and plasma concentration–time profiles after subcutaneous (s.c.) implantation.

EXPERIMENTAL

Materials

Chitosan with a degree of deacetylation of 56% [viscosity = 17–25 cp at 1% (w/w) in 1% (w/w) aqueous acetic acid at 20°C] was supplied from Dainichi-Seika Color & Chemicals Mfg. Co., Ltd. (Japan). Dow Corning 360 Medical Fluids of 100 cs (25°C) and 350 cs (25°C) were purchased as silicone oils from Dow Corning Co. (USA). 5-Fluorouracil was supplied from Teijin Co. (Japan). Tegafur was purchased from Aldrich Chemical Company, Inc. (USA). Doxifluridine was supplied by Nippon Roche Co., Ltd. (Japan). Glutaraldehyde was purchased as a 25% aqueous solution from Sigma Chemical Co. (USA). 5-Iodouracil was purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan). Olive oil was purchased from Iwaki Seiyaku Co., Ltd. (Japan).

Preparation of Microspheres

Medical Fluids of 100 cs (25°C) and 350 cs (25°C) were mixed to get a viscosity of 250 cs (25°C). This silicone oil mixture with 250 cs (25°C) was used as an oil phase in preparation of microspheres.[15] Chitosan (1 g) was dissolved at a concentration of 5% (w/v) in 1% (v/v) aqueous acetic acid solution. Drugs were dissolved in the chitosan solution at various drug/chitosan ratios of 10–50% (w/w). The chitosan solution containing the drug (25 mL) was put drop-wise into 500 mL of the above silicone oil mixture [250 cs (25°C)], and stirred at 500 rpm under reduced pressure using an aspirator for 24 h. The water-in-oil system was warmed from room temperature to 50°C,

and the stirring at 500 rpm under reduced pressure using the aspirator was continued for 24 h. Then, pressure reduction was performed with a vacuum pump under the stirring at 500 rpm at 50°C to dry the produced microspheres completely for 24 h. The microspheres were separated by filtration of the preparation mixture, and washed with ether. The microspheres were washed quickly with 10% (w/v) sodium hydroxide aqueous solution, and then washed with water and ethanol in that order. The microspheres were dried in a desiccator, and sieved with 12-, 30-, and 48-mesh sizes (JP XIII).

Reacetylation and Coating with Chitosan

Reacetylation was performed for DFUR-containing microspheres (DFUR-M) according to Li et al.[12] Briefly, DFUR-M (1 g) was put in the mixture of 10 mL methanol and 30 mL acetic anhydride and kept for 1 h at room temperature. After that, the microspheres were taken with a filter, washed with water, ethanol, and ether in that order, and dried in air. The obtained microspheres were expressed as DFUR-M reacetylated in the presence of methanol. Also, reacetylation with no addition of methanol was executed, that is, the microspheres (1 g) were treated in the same manner as above except that only acetic anhydride (30 mL) was used instead of the above mixture of methanol and acetic anhydride. The obtained microspheres were expressed as DFUR-M reacetylated in the absence of methanol.

Chitosan coating was also performed for DFUR-M as follows: The microspheres were immersed into 5% (w/v) chitosan solution in 1% (v/v) aqueous acetic acid, taken out by filtration, treated with glutaraldehyde for 10 s, washed with water, and dried. The obtained microspheres were expressed as DFUR-M coated with chitosan alone. Also, coating of the microspheres was executed in the same manner as above except that the 1% (v/v) aqueous acetic acid containing 5% (w/v) chitosan and 35% (w/v) DFUR was used instead of 1% (v/v) aqueous acetic acid containing 5% (w/v) chitosan. The obtained microspheres were expressed as DFUR-M coated with chitosan plus drug.

Particle Size and Shape and Drug Content

The particle size of the microspheres was examined by an Olympus-SZH stereoscopic microscope equipped a camera. The Green diameters of 200 particles randomly chosen from the photomicrograph were measured. The mean particle diameter was determined from a median diameter of the cumulative curve of the particle diameters.

The particle shape of the microspheres was observed using a JEOL JSM-T200 scanning electron microscope after coating the microspheres with a gold layer of about 200 Å in thickness, and their photomicrographs were taken using the equipped camera.

The drug content was measured as follows. First, 10 mg microspheres were put into 10 mL water and kept at 37°C for 3 h. Then, the microspheres were mashed by treating the aqueous suspension with a glass homogenizer. The mixture was then diluted to 50 or 100 mL with the addition of water. After filtration of this sample, the drug content of the microspheres was determined from the absorbance of the filtrate at 265, 271, and 269 nm for 5-FU, FT, and DFUR, respectively.

In Vitro Drug Release

The drug release test was performed for nontreated, chitosan-coated and reacetylated DFUR-M, which were prepared at the DFUR/chitosan ratio of 10% (w/w). First, 10 mg microspheres were put in 100 mL JP XIII second fluid (pH 6.8). Immediately after that, the mixture was stirred at 37°C at 200 rpm using a magnetic stirrer. At appropriate times, aliquot samples (each 5 mL) were withdrawn, and the fresh media (each 5 mL) were supplemented immediately after sampling. Each sample was measured spectrophotometrically at 269 nm to determine the amount of released DFUR.

In Vivo Degradation

The in vivo degradation study was performed for nontreated DFUR-M and DFUR-M reacetylated in the presence of methanol, which were prepared at the DFUR/chitosan ratio of 10% (w/w). First, 50 mg microspheres were suspended in a small amount of olive oil. Then, the mixture was subcutaneously injected using an implantation syringe (2 mm ϕ × 35 mm length) into the back of each male BDF_1 mouse (6 weeks old), which was purchased from Clea Japan Inc. At an appropriate time point after subcutaneous (s.c.) implantation, the mouse was sacrificed, and all the remaining microspheres were

collected from the implantation site and washed with purified water, acetone, and chloroform in that order, which gave the microspheres washed well in good recovery.[15] The microspheres were dried in vacuo and weighed. The degraded amount of the microspheres was determined from the weight decrease.

The microspheres recovered in the in vivo degradation study were observed with a scanning electron microscope in the same manner as described above in the investigation of particle shape. Their photomicrographs were taken for the microspheres recovered at 14 and 28 days after implantation.

Plasma Concentration–Time Profile After S.c. Implantation

Male Wistar rats (160–180 g) were anesthetized by intraperitoneal injection of urethan saline solution at 0.75 g/3 mL/kg and fixed on the back. Nontreated DFUR-M, prepared at the drug/chitosan ratio of 10% (w/w), and DFUR-M reacetylated in the presence of methanol, prepared at the drug/chitosan ratio of 50% (w/w), were used in this experiment. The microspheres were implanted subcutaneously into the back of a rat at the dose of 25 mg DFUR eq./kg. At appropriate time points, blood (0.7 mL) was taken from the jugular vein using a heparinized syringe. Plasma was obtained by centrifugation. To 0.3 mL plasma, 3 μg 5-iodouracil (internal standard), 50 μL of 1 M phosphate buffer (pH 6), and 8 mL ethyl acetate were added in that order. The mixture was shaken for 10 min and centrifuged, and then 7 mL of organic phase was taken and dried at 45°C under nitrogen gas. The residue was dissolved in the solution of water–methanol (49:1, v/v), and filtered with a membrane filter (pore size 0.22 μm). The filtrate was analyzed by high-performance liquid chromatography (HPLC), which was performed at room temperature according to the method by Machida et al.[29] Briefly, 50 μL of the sample was injected on a YMC PACKED COLUMN A-312. A linear gradient method using a mixture of water and methanol (49:1, v/v) (mobile phase A) and a mixture of water and methanol (4:1, v/v) (mobile phase B) was applied to the assay at a flow rate of 1.4 mL/min. The mobile phase A was applied for 0–1 min, the flow was gradually changed in the linear manner from the mobile phase A to the mobile phase B for 1–12.5 min, and then the mobile phase B alone was used from 12.5 min. The detection was done spectrophotometrically at 266 nm. The concentration of DFUR was determined using 5-iodouracil as an internal standard.

Statistical Analysis

Statistical analysis was performed using the unpaired *t*-test. Significant difference was set as $p < 0.05$.

RESULTS AND DISCUSSION

Particle Characteristics

As reported previously,[15] chitosan mirospheres could be obtained well when silicone oil (specific gravity = 0.97) with no surfactant was used as an oil phase in the dry-in-oil method. Microspheres, however, could not be obtained due to aggregation when using liquid paraffin (specific gravity = 0.87) with no surfactant as an oil phase. That is, when using silicone oil as an oil phase in the dry-in-oil method, microspheres can be obtained because high specific gravity and high viscosity of silicone oil prevent aggregation of microspheres. The microspheres obtained using silicone oil as an oil phase are purely composed of chitosan and drug because no surfactant is used in the preparation. In the present study, the dry-in-oil method using silicone oil as an oil phase was applied to preparation of chitosan microspheres containing 5-FU-related antitumor agents (5-FU, FT, and DFUR). As a resut, microspheres with a fairly large particle diameter were obtained as depicted in Fig. 1. The particle size was similar to that of the microspheres composed of chitosan and *p*-hydroxybenzoate esters, reported previously.[15] The particle size became larger with the increase in drug/chitosan ratio. The mean particle diameter was approximately 300–400 μm under the drug/chitosan ratio of 10% (w/w) for all the microspheres. Further, the size distributions were similar. The 5-FU-containing microspheres tended to show a larger particle size than other microspheres with the addition of higher drug amount. The microspheres containing 5-FU could not be obtained at the drug/chitosan ratio of 50% (w/w) because of their aggregation; therefore, the result at the drug/chitosan ratio of 50% (w/w) is not shown for 5-FU-containing microspheres (Fig. 1). The micropsheres containing FT and DFUR exhibited similar relationships between amount of the added drug and particle size. Non-treated DFUR-M and DFUR-M reacetylated

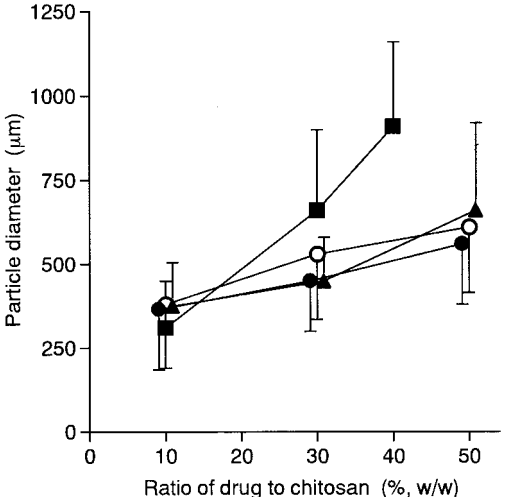

Figure 1. Particle size and its distribution of microspheres prepared at different drug/chitosan ratios: ■ 5-FU; ▲ FT; ● DFUR; ○ DFUR (reacetylated in the presence of methanol). The results are expressed as the median diameter ± square root of the mean value of square of deviation from the median diameter for 200 microspheres.

in the presence of methanol also showed similar relationships between amount of the added drug and particle size.

Figure 2 shows the drug content of nontreated microspheres after sieving with some meshes. Irrespective of the size of microspheres, the drug content became higher with the increase in drug/chitosan ratio. Smaller microspheres tended to show less drug content. However, such dependence of drug content on the particle size was small. DFUR-containing microspheres exhibited the highest drug content. Their drug contents were 6–7% (w/w) and 20–22% (w/w) at the drug/chitosan ratios of 10 and 50% (w/w), respectively. The microspheres containing 5-FU and FT showed lower and similar drug content.

The ideal drug content was calculated as a ratio of the amount of added drug to the total amount of added chitosan and added drug. The encapsulation efficiency was calculated as a ratio of observed drug content to ideal drug content. The result is shown in Fig. 3. The encapsulation efficiency was relatively high. The encapsulation efficiencies were higher in the order of DFUR > 5-FU > FT at each drug/chitosan ratio. DFUR-M exhibited the encapsulation efficiency of approximately 75% at the drug/chitosan ratio of 10% (w/w).

As stated above, the drug content and encapsulation efficiency were highest in DFUR. The partition

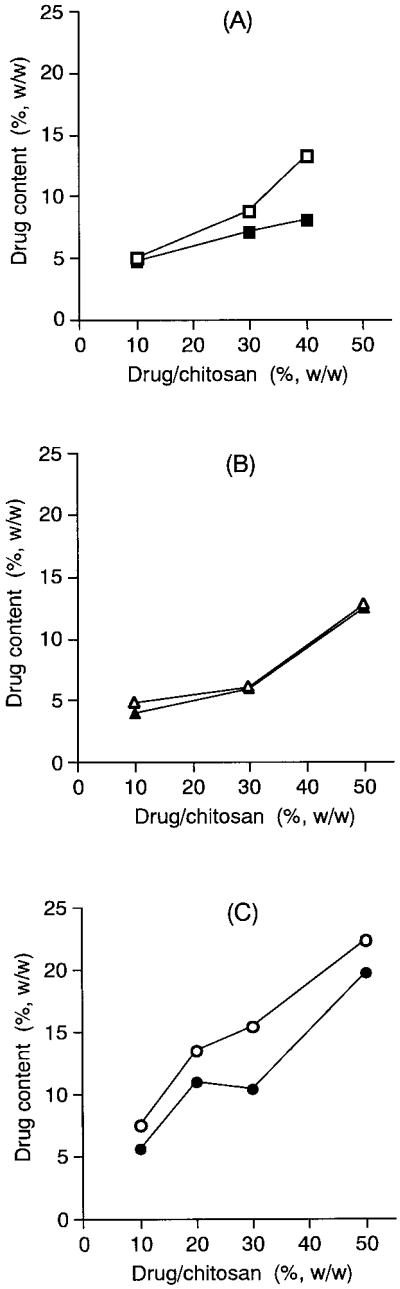

Figure 2. Drug content of microspheres sieved with meshes after preparation at different drug/chitosan ratios: (A) 5-FU; (B) FT; (C) DFUR. ○ Open symbols represent the microspheres with diameters of 12–30 mesh size; ● Closed symbols represent the microspheres with diameters of 30–48 mesh size.

coefficient in (silicone oil)/[1% (v/v) aqueous acetic acid] was in the order of FT > 5-FU > DFUR. The partition coefficient of DFUR was less than 0.05, which was 1/3 of that of 5-FU and less than 1/30 of

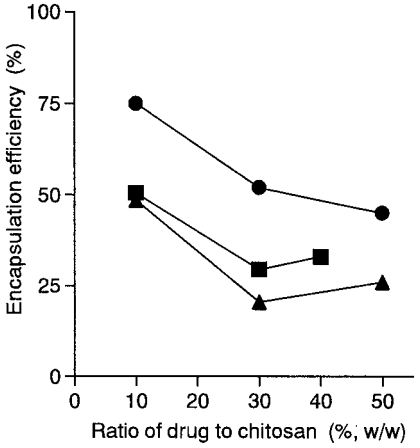

Figure 3. Encapsulation efficiencies of microspheres prepared at different drug/chitosan ratios: ■ 5-FU; ▲ FT; ● DFUR. The encapsulation efficiency was calculated as (observed drug content)/(ideal drug content).

that of FT. Therefore, it was suggested that FT and 5-FU should transfer more easily to the oil phase and DFUR should be retained better in the microspheres, resulting in the high drug content and high encapsulation efficiency in DFUR. The drug precipitation was recognized on the particle surface at the drug/chitosan ratio of 50% (w/w) in DFUR and at that of 30% (w/w) in 5-FU and FT, respectively, indicating that a larger amount of the drug transferred out of the surface more easily at the higher drug/chitosan ratio. Thus, encapsulation inside the microspheres appeared to be insufficient at the higher drug/chitosan ratio, which might lead to the lesser encapsulation efficiency at the higher drug/chitosan ratio.

Chemical stabilities of 5-FU, FT, and DFUR were investigated under the same conditions as described in the preparation of the microspheres. Namely, each drug was dissolved in 1% (v/v) aqueous acetic acid solution, and stirred for 1 day at room temperature and subsequently for 2 days at 50°C. Then, each drug solution was lyophilized. Each drug after treatment was analyzed using ^{1}H-NMR spectra and HPLC. As a result, decomposition was not recognized for 5-FU and DFUR, but FT was found to be degraded into 5-FU to some extent. Therefore, for FT-containing microspheres, the drug content obtained above was regarded as an apparent value, that is, FU was considered to coexist to some extent in FT-containing microspheres.

For all the microspheres prepared with the DFUR/chitosan ratios of 10, 30, and 50% (w/w), more than 60% of the incorporated drug was retained after reacetylation in both presence and absence of methanol. Figure 4 shows scanning electron micrographs of nontreated DFUR-M, DFUR-M reacetylated in the presence and absence of methanol, and DFUR-M coated with chitosan alone, all of which were prepared with addition of 10% (w/w) DFUR/chitosan. Nontreated and reacetylated DFUR-M possessed a smooth surface. On the other hand, chitosan-coated microspheres showed large cracks on the surface. These cracks were considered to be formed by quick solidification of the chitosan-coated layer with glutaraldehyde.

In Vitro Release

The drug release was examined for nontreated, reacetylated, and chitosan-coated DFUR-M, all of which were prepared at the DFUR/chitosan ratio of 10% (w/w). The results are shown in Fig. 5. Nontreated DFUR-M released almost all the drug rapidly. Chitosan-coated DFUR-M suppressed such initial rapid release to some extent. Especially, the initial rapid release was significantly suppressed in DFUR-M coated with chitosan alone, when approximately 25% of the contained drug was released very slowly. Reacetylation of the microspheres in the presence of methanol also significantly suppressed the initial rapid release, in which approximately 30% of the contained drug was released very slowly. Reacetylation in the absence of methanol did not suppress the initial rapid release well. Therefore, presence of methanol might be important in reacetylation, which was consistent with the result by Li et al.[12] For all the microspheres, the drug was released rapidly within the initial 30 min, and then released very slowly. The drug remaining after initial rapid release was expected to be released with the degradation of chitosan matrix.

Thus, modification such as further reacetylation or chitosan-coating or another technique making drug release suppressed was considered necessary to achieve prolonged release sufficiently because initial rapid release was still high in the present modification. The initial release might be suppressed by washing the microspheres more with aqueous solvents such as release medium, though the drug content would be lowered by washing. More detailed examination will be needed for good achievement of the prolonged drug release. Further, the possibility that

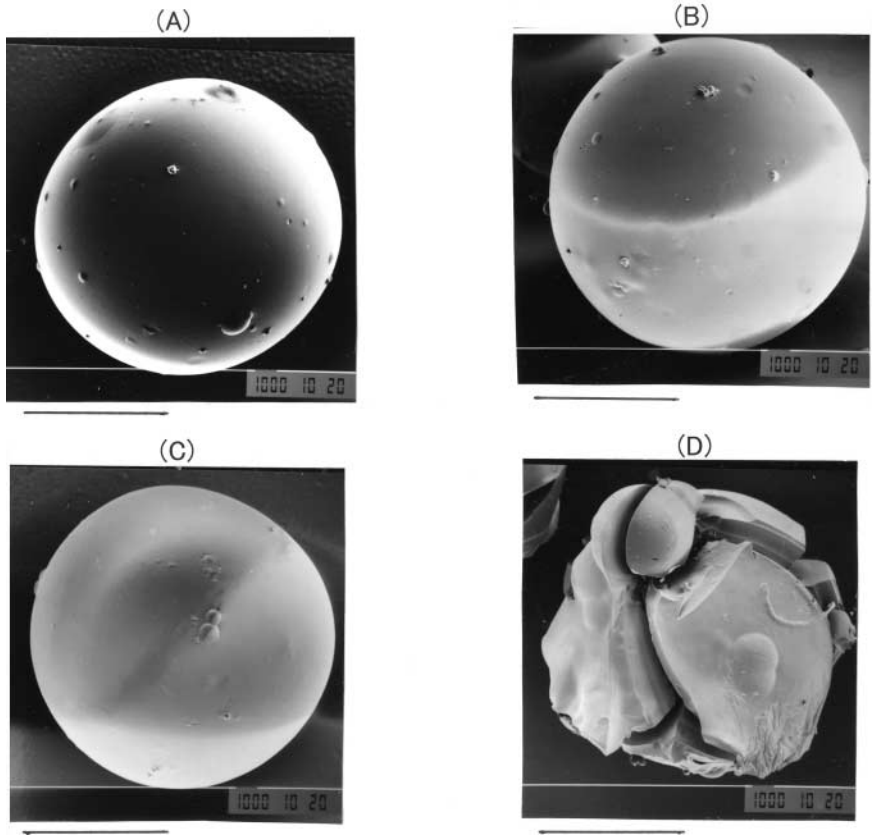

Figure 4. Scanning electron micrographs of nontreated DFUR-M (A), DFUR-M reacetylated in the presence of methanol (B), DFUR-M reacetylated in the absence of methanol (C), and DFUR-M coated with chitosan alone (D): all microspheres were prepared at the drug/chitosan ratio of 10% (w/w). The magnifying power, × 75 for all micrographs; the length of the black bar under each photo = 500 μm.

treatment with acetic anhydride and glutaraldehyde might induce chemical modification of the drug itself will have to be clarified, though sufficient plasma levels of DFUR were observed in reacetylated DFUR-M as described in the following in vivo study (Plasma Concentration–Time Profile after S.c. Implantation in Rats).

Biodegradation of Microspheres After S.c. Implantation in Mice

This experiment was examined for nontreated DFUR-M and DFUR-M reacetylated in the presence of methanol, all of which were prepared at the DFUR/chitosan ratio of 10% (w/w). The nontreated and reacetylated DFUR-M showed drug contents of 7.5 and 3.9% (w/w), respectively. After s.c. implantation in mice, both nontreated and reacetylated DFUR-M showed rapid loss of 20–30% (w/w) in the initial 1 day, and then degraded slowly (Fig. 6). The in vitro release suggested that DFUR should be released rapidly in vivo (Fig. 5). Also, as the drug contents were considerably low, the remaining drug in the microspheres was not measured in this experiment. In fact, the degradation profiles appeared to be little influenced by the contained drug (Fig. 6). The result indicated that the polymer was lost to some extent from the microspheres in the initial period. Some erosion of the microspheres as well as release of DFUR was considered to occur in the initial time. At 28 days postimplantation, the nontreated and reacetylated DFUR-M underwent biodegradation by 61 and 56% (w/w), respectively. The reacetylated microspheres tended to degrade more slowly than the nontreated microspheres. The introdution of acetyl groups by reacetylation to the original acetylation degree (44%) might reduce the lysozymic degradation of the chitosan matrix. The shapes of the microspheres recovered

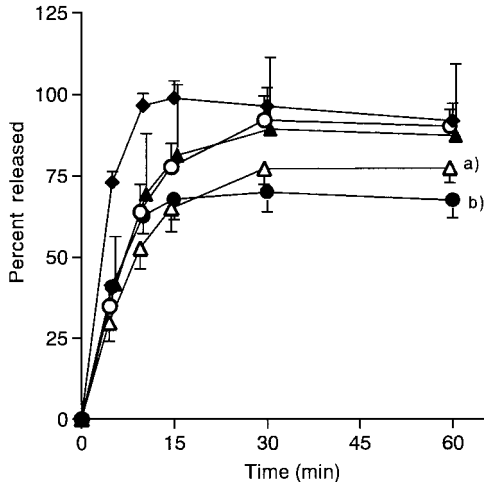

Figure 5. Release of DFUR from microspheres in the JP XIII second fluid (pH 6.8): ◆ nontreated DFUR-M; ▲ DFUR-M coated with chitosan plus DFUR; △ DFUR-M coated with chitosan alone; ○ DFUR-M reacetylated in the absence of methanol; ● DFUR reacetylated in the presence of methanol. All the microspheres were prepared at the drug/chitosan ratio of 10% (w/w). Each point represents the mean ± S.D. ($n = 3$). a) $p < 0.05$ vs. nontreated DFUR-M at 60 min. b) $p < 0.01$ vs. nontreated DFUR-M at 60 min.

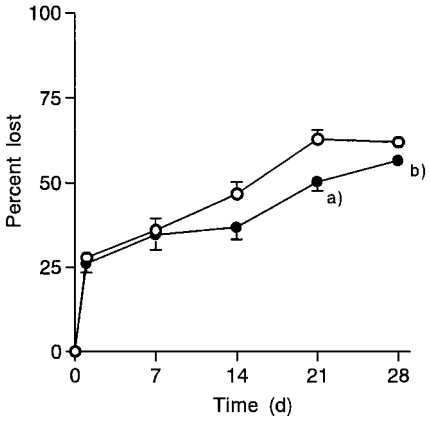

Figure 6. In vivo degradation of microspheres after s.c. implantation: ○ nontreated DFUR-M; ● DFUR-M reacetylated in the presence of methanol. Both microspheres were prepared at the drug/chitosan ratio of 10% (w/w). Each point represents the mean ± S.E. ($n = 3$). a) $p < 0.05$ vs. nontreated DFUR-M at 21 days. b) $p < 0.01$ vs. nontreated DFUR-M at 28 days.

at 14 and 28 days postimplantations are shown in Fig. 7. The microspheres changed into irregular shapes with the progression of degradation. In particular, change in particle shape appeared to be greater in nontreated DFUR-M than reacetylated DFUR-M.

Plasma Concentration–Time Profile After S.c. Implantation in Rats

The plasma concentration–time profiles of DFUR were examined after s.c. implantation of nontreated DFUR-M and DFUR-M reacetylated in the presence of methanol, which were prepared at the DFUR/chitosan ratios of 10 and 50% (w/w), respectively. As blood sampling was performed several times at early time points, rats were used, when consecutive blood sampling could be done in each animal. Both microspheres were implanted at a dose of 25 mg DFUR eq./kg. The nontreated DFUR-M showed a maximum plasma level at 1 h postadministration, while reacetylated DFUR-M showed a maximum concentration at 2 h postimplantation (Fig. 8). However, the plasma concentration was eliminated relatively fast after a maximum concentration in both the microspheres. The plasma levels were not significantly different between both the microspheres at each time point ($p \geq 0.05$). The plasma concentration–time profiles were probably derived from initial rapid release. The plasma level was slight after 5 h postimplantation, which was proposed to be due to very slow drug release after initial burst. However, as reacetylated DFUR-M prepared at the drug/chitosan ratio of 50% (w/w) was not investigated for drug release, examination of the drug release will be required for more exact evaluation of these plasma concentration–time profiles. Also, the plasma concentration–time profile of DFUR-M reacetylated in the presence of methanol, prepared at the drug/chitosan of 10% (w/w), will be required to be examined for direct comparison with nontreated DFUR-M.

Furthermore, the similar plasma levels of DFUR in both nontreated and reacetylated DFUR-M also suggested that reacetylation might not influence the drug itself. Namely, reacetylation of the microspheres was considered not to impair DFUR itself or availability of DFUR.

These plasma concentration–time profiles indicated that the microspheres tested could not fulfill the prolonged retention of DFUR as well as the results in the in vitro release. Rapid initial release will have to be suppressed much more for in vivo prolonged release. The similar plasma levels of DFUR in both nontreated and reacetylated DFUR-M also suggested that reacetylation should not influence the drug itself.

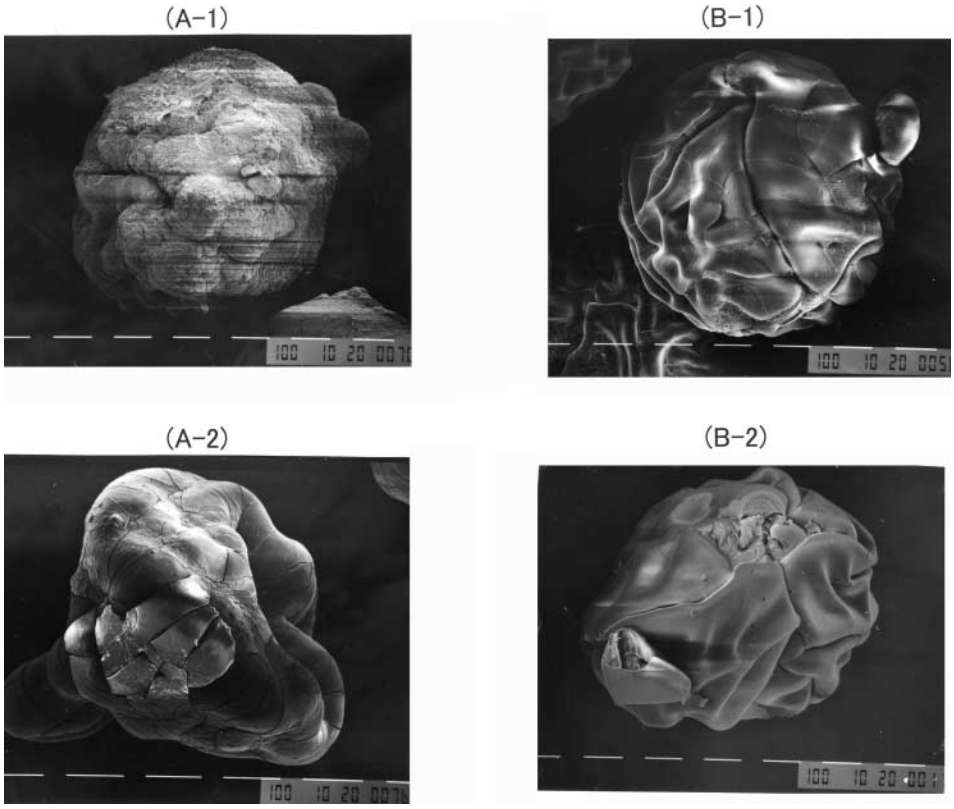

Figure 7. Scanning electron micrographs of microspheres recovered at 14 and 28 days after s.c. implantation: (A-1) nontreated DFUR-M, 14 days postimplantation; (A-2) nontreated DFUR-M, 28 days postimplantation; (B-1) DFUR-M reacetylated in the presence of methanol, 14 days postimplantation; (B-2) DFUR-M reacetylated in the presence of methanol, 28 days postimplantation. All microspheres were prepared at the drug/chitosan ratio of 10% (w/w). The length of the white bar in each photo = 100 μm.

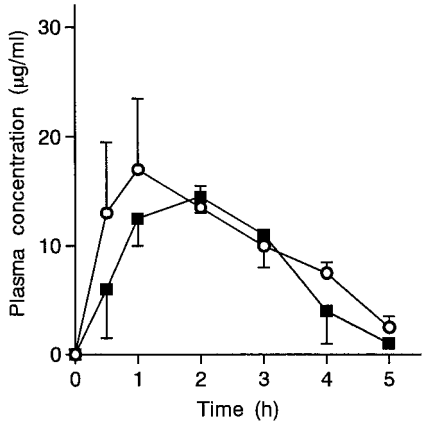

Figure 8. Plasma concentration–time profiles of DFUR after s.c. implantation of microspheres: ○ nontreated DFUR-M prepared at 10% (w/w) drug/chitosan ratio; ■ DFUR-M prepared at 50% (w/w) drug/chitosan ratio and reacetylated in the presence of methanol. Each point represents the mean ± S.E. ($n = 3$ for nontreated DFUR-M; $n = 4$ for reacetylated DFUR-M).

CONCLUSION

In the present study, the dry-in-oil method using silicone oil with no surfactant as a dispersion medium was applied to the preparation of chitosan microspheres containing 5-FU, FT, and DFUR. The obtained microspheres exhibited mean diameters of 300–900 μm and showed relatively high drug contents. DFUR-containing microspheres did not exhibit a prolonged drug release in vitro, but modification techniques such as reacetylation or chitosan coating allowed the suppression of the initial rapid release to some extent. However, initial rapid release was not improved essentially. DFUR-containing microspheres underwent gradual biodegradation after s.c. implantation. However, prolonged retention of the plasma level of DFUR was not observed after s.c. implantation of DFUR-containing microspheres. Thus, biodegradable chitosan microspheres containing DFUR with a fairly large particle size and good

drug content could be obtained by the present dry-in-oil method, but their drug release features remain to be improved.

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