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Preparation of polymeric nanoparticles of cyclosporin A using infrared pulsed laser

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ABSTRACT

Nanoparticle formation of poorly water-soluble drugs is a means of providing much benefit for improving solubility and bioavailability. We showed that laser irradiation of drugs can be a novel tool for dispersing drug nanoparticles in water. Using our method, we were able to produce nanoparticles containing immunosuppressant drug, cyclosporin A, which shows poor solubility toward water, with high levels of the drug using polyvinyl pyrrolidone and sodium dodecyl sulfate as stabilizing agents. The absence of degradation products was confirmed and the loss of pharmaceutical activity with an inhibitory effect on the interleukin-2 production of Jurkat T cells did not occur. Cyclosporin A nanoparticles showed a spherical shape and their particle size was distributed uniformly around 200 nm. Powder X-ray diffraction analysis suggested that cyclosporin A in the nanoparticles was in an amorphous state. In the measurement of solubility rate, the nanoparticle formulation showed a higher rate than that which had not been processed. At present, although this laser irradiation technology has low productivity, it is expected as a new technology for drug nanoparticle manufacturing together with the development of a new laser device.

1. Introduction

The search for new drugs is difficult because many candidate compounds have poor solubility in water (Lipinski, 2000; Kola and Landis, 2004). Low solubility of drugs has a great influence on pharmacokinetic and pharmacodynamic properties and leads to low bioavailability (Kipp, 2004; Prentis et al., 1988). The improvement of water solubility is an extremely important task for pharmaceutical companies in developing new medicines.

There are some approaches to improve the solubility of poorly water-soluble drugs, for instance, salt generation, cocrystal formation or complexation with cyclodextrins (Akers, 2002). Amorphous state formation by making a solid dispersion is also effective to elevate the apparent solubility (Serajuddin, 1999; Craig, 2002; Yu, 2001). It is well known that little difference in blood drug levels is seen between the fasting and satiety states when administering an amorphous formulation. At the same time, various technologies for drug refinement processes have been developed in recent years. In particular, there has been a strong interest in nanoparticle dosage forms for improving water solubility and membrane permeability (Merisko-Liversidge et al., 2003; Rawat et al., 2006). According to Noyes–Whitney and Ostwald–Freundlich equation, particle size in the nanometer range can lead to increased dissolution rate and saturation solubility (Hintz and Johnson, 1989; Böhm and Müller, 1999). Many processes of manufacturing nanoparticles are known and these are classified into two main groups: dry and wet approaches. Using a dry process, for example, grinding with mortar and pestle, rod milling or jet milling, it is possible to manufacture small particles with up to 1 μm diameter (Kondo et al., 1993). Manufacturing with a wet process, for example, ball milling or using a high-pressure homogenizer, the particle diameter of the drug is supposed to reach the sub-micron level because the solvent acts as a fracturing agent (Itoh et al., 2003; Kondo et al., 1993). However, in processing pharmaceuticals, there are various problems with regard to the stability and homogeneity of the processed drug. In particular, external or internal contamination is an important issue. Handling in the milling process is well known to cause contamination of the grinding media, and completely preventing contamination is very difficult (Muller and Keck, 2004). Furthermore, some drugs cannot be easily handled with a high-pressure homogenizer owing to the focal elevation of water temperature, which induces a degradation process of organic compound sensitive to heat (Mehnert and Mäder, 2001).

In this report, we describe a laser technique to disperse drug nanoparticles in a wet manner. There has been an excellent study in which the use of laser irradiation for nanoparticle formation with some organic dyes and an anticancer drug was attempted (Asahi et al., 2008). Characteristics of our method here are fixation of the target drug at a focal spot of laser irradiation and the use of a pulsed

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laser with wavelengths at the near-infrared region. Near-infrared light is insufficient to induce electronic transitions like ultraviolet light; therefore, it is considered that drug molecules could not be degraded. Using suppressed heat generation via a pulsed laser, there is also a significant advantage that thermal degradation is less likely. Several studies using laser irradiation methods have already been reported (Nagare and Senna, 2004; Nagare et al., 2006) and some venture firms and companies have developed laser technology for drug manufacturing in recent years. These new laser methods are expected to have great potential for manufacturing drug nanoparticle formulations.

The target drug for nanoparticle formation in the present study is cyclosporin A (CsA). CsA is a potent immunosuppressant used primarily to reduce the incidence of graft rejection in recipients of transplanted organs and to treat systemic and local autoimmune disorders (Cohen et al., 1984; Dunn et al., 2001; Thomson and Neild, 1991). It is a neutral cyclic peptide with high molecular weight and high lipophilicity, showing very low water solubility (about 20 μg/mL) (Miyake et al., 1999; Aliabadi et al., 2005). The product on the market today is a self-emulsifying formulation (Sandimmun® and Neoral®) comprised of ethanol and polyoxyethylated castor oil (Mueller et al., 1994); however, it is suggested that this liquid formulation has significant problems with regard to side effects (Gelderblom et al., 2001). To improve the efficacy of CsA and reduce its side effects, alternative formulations have been achieved, including biodegradable polymeric micelles (Aliabadi et al., 2005; Zhang et al., 2009), solid lipid nanoparticles (Müller et al., 2006), pH-sensitive nanoparticles (Dai et al., 2004) and positively charged nanoparticles (El-Shabouri, 2002). We used here a water-soluble polymer, polyvinyl pyrrolidone (PVP), and a surfactant, sodium dodecyl sulfate (SDS), as dispersion stabilizers and succeeded in preparing polymeric nanoparticles of CsA with high levels of the drug. It is considered that PVP inhibits the crystallization of drug and SDS contributes to the stability of nanoparticles by producing a negative charge on the surface (Itoh et al., 2003). The preparation method using laser technology and the physicochemical properties of the prepared CsA nanoparticles are investigated in this study.

2. Material and methods

2.1. Materials

CsA and PVP K30 were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used as received. SDS was purchased from Sigma (St. Louis, MO). Other chemicals and solvents were of analytical and high-performance liquid chromatography grade. Milli Q Plus ultrapure water (Millipore, Billerica, MA) was exclusively used for the preparation of aqueous solutions and nanoparticle dispersions.

2.2. Preparation of nanoparticles

2.2.1. Nanoparticle formation by laser irradiation

CsA-loaded polymeric nanoparticles were prepared as follows. The manufacturing process was divided into two steps.

First, 5 mg of CsA bulk powder, 25 mg of PVP and 1 mg of SDS were placed in a test tube and dissolved in 500 μL of ethanol. The ethanol was dried under reduced pressure conditions to obtain a mixture containing the drug and the dispersion stabilizers homogeneously dispersed.

Then, the mixture was hermetically sealed upon adding 500 μL of water. Neodymium-doped yttrium aluminum garnet (Nd:YAG) pulsed laser light was irradiated from a side of the test tube onto the mixture inside the test tube (Fig. 1). The irradiation conditions were as follows: 1064 nm wavelength, 0.61 J/cm^2/pulse irradiation light intensity, 5–7 ns pulse width and 10 Hz repetition frequency. After 10 min of irradiation at room temperature, almost all of the mixture contained in the tube was dissipated. Unless otherwise noted, these operations were carried out at room temperature (25 °C). When processed in 4 °C, the test tube was covered with cooled N_2 gas.

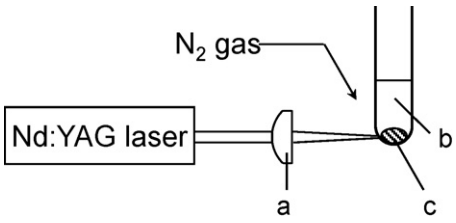

Fig. 1. Schematic of apparatus used for laser irradiation through lateral side of the glass tube. a: lens, b: water, c: mixture including drug and excipients.

2.2.2. Nanoparticle formation by ball milling

Drug and excipients were milled at room temperature (25 °C) using a planetary micro mill (Pulverisette 7, FRITSCH, Idar-Oberstein, Germany). 40 mg of CsA, 200 mg of PVP, 8 mg of SDS and 4 mL of water were measured in a mixing chamber and rotated at 500 rpm for 1 h with 0.6-mm-diameter zirconium oxide beads.

2.3. Analysis of pharmacological activity of CsA

2.3.1. CsA activity in vitro

The inhibitory effect of CsA on calcineurin phosphatase activity was measured using calcineurin colorimetric drug discovery kit (Biomol, Plymouth Meeting, PA) (Perrino et al., 2002) with a slight modification. In brief, an assay mixture that contained 80 nM calmodulin and 35.6 nM calcineurin was prepared. After further addition of untreated CsA prepared in dimethyl sulfoxide (DMSO) or laser-irradiated CsA diluted in DMSO, cyclophilin A and RII phosphopeptide substrate, they were incubated at 30 °C for 60 min. Biomol Green® reagent, which is colored from yellow to green after reacting with free phosphate, was added and then OD_{620} was measured.

2.3.2. Inhibitory effect on interleukin-2 (IL-2) production of Jurkat cells

IL-2 release from activated Jurkat cells was measured by a previously reported method (Manger et al., 1986) with a slight modification. In brief, Jurkat cells cultured at 5×10^5/mL were stimulated with phorbol 12-myristate 13-acetate at 50 ng/mL and A23187 at 100 ng/mL in the presence or absence of CsA. After culture for 24 h at 37 °C, IL-2 production in the cell supernatants was measured using an immunoassay kit (R&D Systems, Minneapolis, MN).

2.4. High-performance liquid chromatography (HPLC)

The amount of CsA in nanoparticle dispersion was determined by HPLC (Shimadzu, Kyoto, Japan) as described previously (Wang et al., 2004) with a slight modification. The HPLC system was comprised of Shimadzu LC-10AD pump, CTO-10A column oven and SPD-M10A detector set at 210 nm. A reverse-phase ODS-80Ts C18 column (4.6mm × 150 mm, Tosoh, Tokyo, Japan) was used to detect CsA under incubation at 50 °C with a guard column. The mobile phase was acetonitrile/isopropanol/water (55/15/30, v/v/v) and the flow rate was 1 mL/min. The retention time of CsA in the set condition was around 10.5 min.

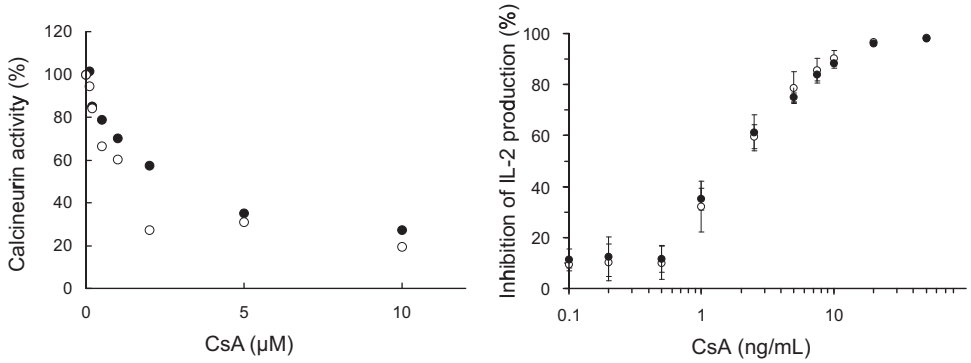

Fig. 2. (A) Inhibition of calcineurin activity by CsA. Calcineurin activities are expressed as percentages of the activity in the absence of CsA. (●) Non-treated CsA and (○) irradiated CsA in DMSO solution. (B) Effects of CsA on IL-2 production from Jurkat cells stimulated with PMA/A23187. (●) CsA solution in EtOH and (○) aqueous suspension including CsA nanoparticles. Each point represents the mean ± SD ($n = 3$).

2.5. Morphological characterization

The morphology of the nanoparticles was investigated by scanning electron microscopy (S-4200, Hitachi High-Technologies, Tokyo, Japan) at an accelerating voltage of 10 kV. Samples were prepared on a 200-nm-pore filter and desiccated under dry air prior to examination.

2.6. Particle size distribution

The particle size and size distribution were determined using a laser diffraction particle size analyzer (SALD-7000, Shimadzu, Kyoto, Japan). The diameter was calculated from the intensity pattern of the scattered light passed through the particles assuming a spherical form with guessed optical parameters (real refractive index: 1.70, imaginary refractive index: 0.1) (Keck, 2010).

2.7. Determination of encapsulation efficiency

Free CsA content in the external aqueous phase was determined by centrifuging 1 mL of nanoparticle dispersion at 100,000 × *g* for 1 h. After centrifugation, CsA amount extracted from the clear supernatant was determined by HPLC. The encapsulation efficiency was calculated by subtracting the free drug concentration from the total concentration found in the dispersion.

2.8. Powder X-ray diffraction analysis

The nanoparticle dispersion was flash-frozen with liquid nitrogen and lyophilized to produce dry powder. The crystallinity of the dry powders was examined with a Rigaku X-ray diffractometer (RINT 1100, Rigaku, Tokyo, Japan) using Cu Kα radiation ($\lambda = 0.1541$ nm) at 30 kV and 10 mA.

2.9. In vitro CsA release

CsA-loaded nanoparticles in lyophilized powder were placed in a meshed cage in a dissolution test apparatus (NTR-1000, Toyama Sangyo, Osaka, Japan). The release media contained 0.1% Tween 80 as a solubilizer. The assay mixture was incubated at 37 °C with continuous orbital mixing (100 rpm). At specified time intervals (1, 2, 3, 5, 10, 15, 20, 30, 60, 90, 120 and 180 min), 1 mL of the supernatant was removed using a syringe fitted with 0.2-μm-pore polycarbonate membrane (Millipore, Billerica, MA). CsA concentrations in the aliquots were analyzed using HPLC. The cumulative amount of CsA release from the nanoparticles was calculated and plotted versus time. Each data point was the mean (±SD) calculated from three measurements.

3. Results

3.1. Preparation of CsA nanoparticles without destruction of intramolecular bonds

The scheme of the apparatus is shown in Fig. 1. Optical conditions were optimized for CsA nanoparticle formation, as shown in Section 2. CsA and the excipients in the form of a mixture were fixed inside a test tube at a focal spot. As shown in Fig. 1, laser light was first irradiated at the mixture and then at water. White-colored particles were produced gradually at the interface of the mixture and water. By shaking gently after laser irradiation, a uniformly cloudy dispersion liquid was obtained. CsA amount contained in the dispersion was assessed using HPLC. The average amount of CsA in the prepared dispersion was found to be 9.15 ± 0.32 mg/mL ($n = 3$). Intact CsA was eluted at a position of approximately 11 min, and no increase in impurity peaks due to laser irradiation was seen in the HPLC diagram. The rest of CsA not recovered in nanoparticles was thought to be remained in the form of a mixture. To assess whether CsA nanoparticles retained pharmacological activity, the inhibitory effect of CsA on calcineurin phosphatase activity was measured. Phosphatase activity of calcineurin was assayed by determining the extent of free-phosphate release from a phosphopeptide substrate in the absence or presence of CsA nanoparticles. The phosphatase activity was found to be potently inhibited by the addition of CsA in a concentration-dependent manner (Fig. 2A). The inhibitory effect of CsA nanoparticles was almost the same as that of the CsA solution prepared in DMSO. From this result, it was confirmed that CsA nanoparticles retain pharmacological activity *in vitro*. Next, we examined whether CsA nanoparticles inhibit IL-2 production in activated T cells. IL-2 transcription requires intact function of a transcription factor, NFAT (nuclear factor of activated T cells), and this factor is activated by calcineurin through dephosphorylation (Andrus and Lafferty, 1981). Therefore, inhibition of calcineurin activity by CsA leads to the prevention of the transcription of IL-2. To determine if *in vivo* inhibition of CsA on IL-2 production from T cells occurred, Jurkat cells derived from a human T cell leukemia were used in this experiment. Compared with the ethanol solution, CsA nanoparticle dispersion had equivalent inhibitory effect on IL-2 production in a concentration-dependent manner (Fig. 2B). In the above *in vitro* and *in vivo* studies, it was confirmed that no denaturing process occurred during the laser irradiation.

3.2. Characterization of CsA nanoparticles

Fig. 3 is a diagram of the particle size distribution of the CsA nanoparticles in the dispersion. The dispersion obtained by laser irradiation at room temperature (25 °C) had a major peak around

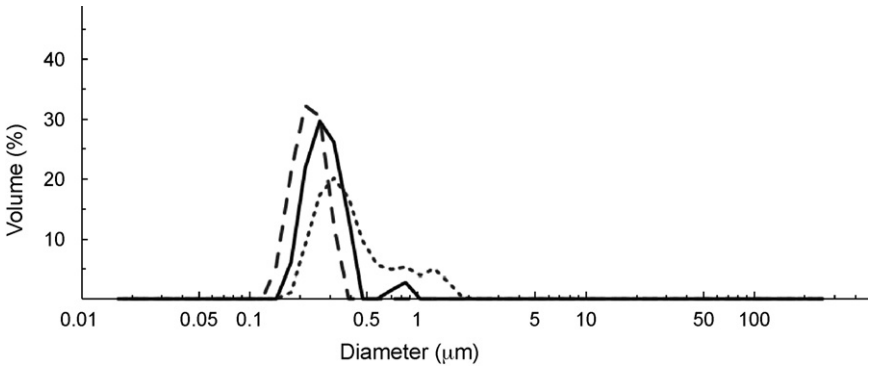

Fig. 3. Comparison of particle size distribution of CsA nanoparticle dispersion prepared by laser irradiation at 25 °C (continuous line) and at 4 °C (dashed line) and by ball milling (dotted line).

250 nm and a relatively small peak at 800 nm. On the other hand, at 4 °C, the distribution of diameter was slightly lower than that of the nanoparticles prepared at 25 °C, and had a peak at 200 nm. From these results, it seems to be possible to prepare nanoparticle dispersions with different particle diameters by varying the temperature of the liquid phase during laser irradiation. Comparative analysis with the nanoparticles prepared by ball milling, generally used for nanoparticle manufacturing, clearly showed a difference. Ball milling products had a wide and relatively higher distribution. Table 1 shows the median diameter, 90%D and encapsulation efficiency from the nanoparticle dispersion prepared by using these techniques. The median diameter obtained for the ball milling products was relatively close to that of the laser-irradiated products, whereas the 90%D, which is the equivalent diameter where 90 mass % of the particles have a smaller diameter, was much higher (391 nm < 1018 nm). This may be explained by the difference of the mechanism of processing nanoparticles. Preparation of nanoparticles by laser irradiation included a process forming the miscible

Table 1
Particle size and encapsulation efficiency of CsA nanoparticles.

	Median diameter (nm)	90%D (nm)[a]	Encapsulation efficiency (%)
Laser (25 °C)	274	391	93.84 ± 0.81
Laser (4 °C)	224	296	96.45 ± 0.48
Milling	363	1018	91.94 ± 1.56

[a]Equivalent diameter where 90 mass% of the particles have a smaller diameter.

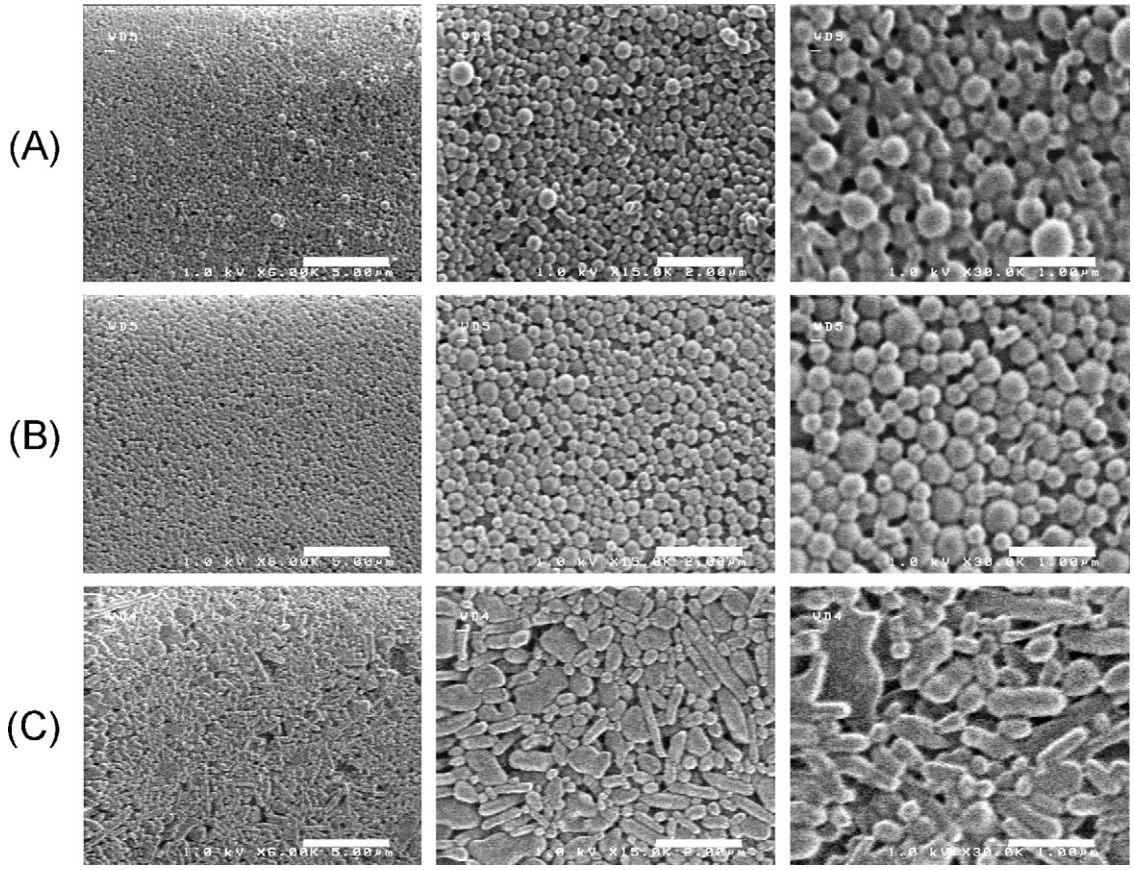

Fig. 4. Scanning electron micrograph images of CsA nanoparticles prepared by (A) laser irradiation at 25 °C and (B) at 4 °C and by (C) ball milling. Scale bar: (left) 5 μm, (center) 2 μm, (right) 1 μm.

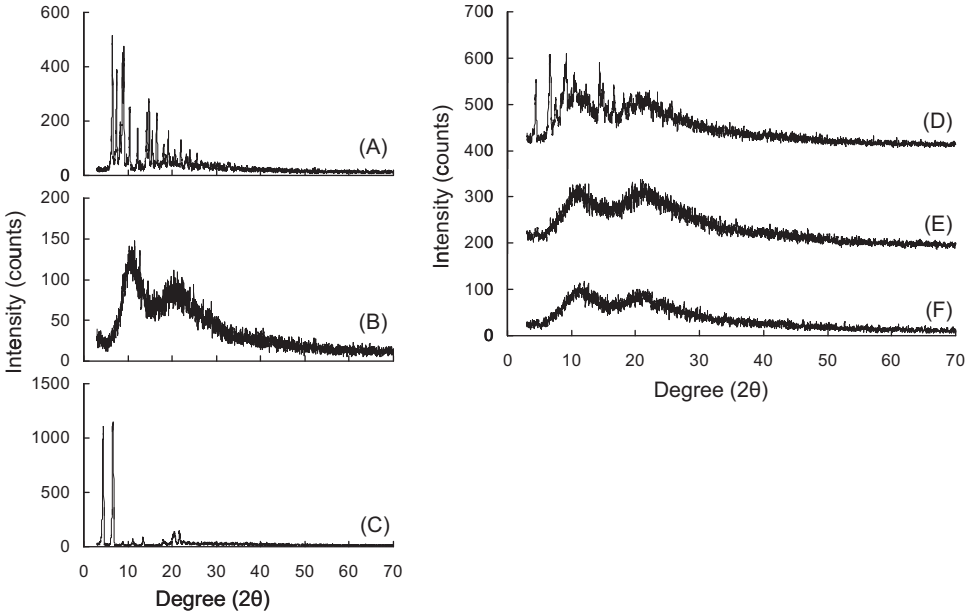

Fig. 5. X-ray diffraction patterns for (A) CsA alone, (B) PVP alone, (C) SDS alone, (D) physical mixture of CsA, PVP and SDS, (E) CsA-loaded nanoparticles processed by laser irradiation and (F) CsA-loaded nanoparticles by ball milling.

drug/polymer mixture highly dispersed at the molecular level. In contrast, the ball milling preparation involved no process of mixing drug and polymer molecularly; as a result, there were small clusters of CsA in some local domains, indicating some level of heterogeneity in the product. To fully disperse the drug molecularly into the polymer is thought to be quite difficult using ball milling technology (Patterson et al., 2007). In relation to the state of drug loaded in the nanoparticles, encapsulation efficiency is also an important factor to determine pharmaceutical properties. All nanoparticle dispersions indicated more than 90% efficiencies. It was considered that almost all CsA added was present in the form of nanoparticles. CsA was thought to moderately interact inside or outside the nanoparticles, so that it seemed difficult to leak into the aqueous phase as a free form.

Fig. 4 is an electron micrograph of the nanoparticles in the dispersion. As can be seen from the micrograph, the nanoparticles obtained by laser irradiation had a spherical shape and a particle diameter of approximately 200–300 nm. These data matched well with the results of the distribution in Fig. 3, and the CsA nanoparticles were thus considered as being a uniform assembly. Laser irradiation process at a lower temperature resulted in the formation of smaller nanoparticles with a narrow size distribution. Ball milling products showed a non-spherical and inhomogeneous shape compared with that of the laser-irradiated products. Particle shape was significantly different between these methods, and that may be also explained by the difference of processing mechanism mentioned above. In each nanoparticle dispersion, sedimentation was hardly noted, even when the dispersion was left to stand at room temperature for one week. Furthermore, lyophilization of the nanoparticle dispersion was thought to be possible. No significant differences in the particle size distribution and the electron microscopy image were seen between the state before lyophilization and a resuspended dispersion liquid (data not shown).

Then, we performed X-ray analysis in order to establish the physical state of both the polymer and the drug in the nanoparticle matrices (Fig. 5). The diffractograms of each pure component were presented separately (Fig. 5A–C), and the diffraction pattern of the physical mixture, which was a simple physical blend of drug and excipients prepared by lightly mixing with a mortar and pestle, showed multiple sharp peaks attributed to crystalline CsA (Fig. 5D). After laser processing, the original crystal structure of the drug disappeared (Fig. 5E). In milled products, we had assumed that crystal phase was possible to be included because the milling process produces nanoparticles by breaking down of bulk crystalline particles, but we could not also confirm any crystal peaks (Fig. 5F). The two broad peaks seen at around 11° and 21° of 2θ were due to the polymer PVP and seemed to have heterogeneity of crystalline state. The absence of crystal peaks in both laser-irradiated and ball-milled nanoparticles indicated that the drug existed in an amorphous state, and we could not clearly show the difference in both the nanoparticles in this test.

Fig. 6 compares the CsA release profiles from the lyophilized nanoparticles made by laser irradiation and ball milling. Compared with a physical mixture, both irradiated and milled products exhibited a fast release rate (below 5 min for 90% of CsA release). Whilst the dissolution rates for these products were similar, the dissolution amount of CsA from laser-irradiated products was higher at

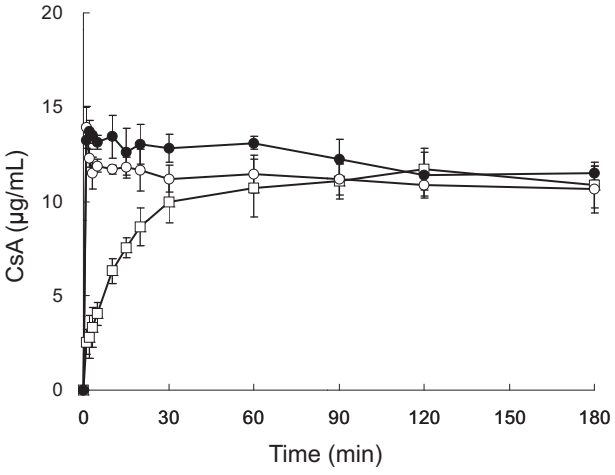

Fig. 6. Dissolution profiles of various formulations of CsA prepared by (□) physical mixture of CsA, PVP and SDS powder, (○) lyophylized nanoparticles prepared after ball milling and (●) lyophilized nanoparticles prepared after laser irradiation. Each point represents the mean ± SD (n = 3).

early stage than that from ball-milled products. This dissolution study was undertaken at a final concentration of 20 μg/mL CsA, so the subtraction of the concentration after equilibrium might be due to the formation of secondary agglomerates, which had been filtrated through 0.2 μm pores. The cause of aggregation occurring more in milled products was thought to be non-uniformity of the particle distribution, especially the existence of large particles over 1 μm, and the heterogeneous particle shape (Figs. 3 and 4). Release amount of the laser product was going down slightly, because the amorphous drug might be transformed partly with time into crystalline state. Finally at 180 min, these dispersions were assumed to reach equilibrium equally in this test condition.

However, the details are not clarified yet, and further investigation is needed to elucidate the property of solid-state CsA nanoparticles.

4. Discussion

Various methods are known for the preparation of nanoparticles of poorly water-soluble drugs. Ball milling in a wet process is used to grind drugs with grinding media into submicron-sized fine particles. A high-pressure homogenizer is also widely used mainly for emulsification by forcing materials in suspension through a very narrow channel or orifice under pressure. The main objective of this research is to evaluate the usefulness of a laser method as a new technology for drug nanoparticle preparation. Compared with the two well-known methods mentioned above, it is clearly different in terms of the energy source for nanoparticle formation being based on laser light. The energy from the laser light is used to produce small particles from its bulk materials. Generally, light irradiation of organic compounds such as drugs is undesirable in terms of quality control because of structural changes and degradation of the components being caused, which may result in changes in efficacy. However, such action is primarily due to light of ultraviolet wavelengths. As we used infrared light at 1064 nm, which is hardly absorbed by almost all organic compounds, drugs were not assumed to degenerate. The energy of infrared light not equivalent to electronic transition energy is considered to be converted to thermal vibration. It is possible for this vibration to induce thermal decomposition of drugs. However, as using a pulsed laser, the real-time light irradiation of the drug is so short that the dispersion temperature rises relatively slowly.

On the one hand, high efficiency is also required for drug nanoparticle preparation. Even if CsA was irradiated as untreated bulked particles flowing in solvent containing excipients, the yield of nanoparticle formation is 10% or less of the whole amount of the drug. Increase of laser intensity or irradiating time had little effect on raising the efficacy. However, when the target of laser irradiation is pretreated to form a homogeneous mixture containing drug and some additives, the efficacy of nanoparticle formation is close to 100%. Therefore, we assume that it is important for increasing the productivity that the drug is fixed at the position of laser irradiation and dispersed molecularly in additives. The additives used in this study, PVP and SDS, have the ability to highly disperse drugs in water. The mixture containing CsA appeared to be readily dispersed in the solvent upon the addition of even a small dose of energy. Ultrasound treatment in sufficient intensity and time could form drug nanoparticles by using this mixture; however, different particle size distribution and particle shapes compared with those of irradiated products were found. It is considered that a different way of giving energy resulted in different properties of the prepared particles. Moreover, usage of different kinds of polymer could induce a change of energy necessary to form nanoparticles from mixture, so that the optical condition would have to be optimized individually. Laser conditions and the ratio of drug to excipients in this report were optimized for CsA nanoparticles, but this method could be applied widely to other drugs. For anti-inflammatory drugs, clobetasone butyrate and indomethacin, widely known as poorly soluble drugs, their nanoparticle dispersions were possible to be prepared under the same conditions as mentioned in Section 2. Microscopic findings indicated that clobetasone butyrate showed a fine spherical shape and had a particle size of around 500 nm. Indomethacin also had homogeneous spherical particles and the particle size was around 300 nm (data not shown). In comparison with different types of drugs including CsA, particle diameter and distribution were quite different, while spherical particle shape was thought to be almost identical. When using this method at the same conditions for various poorly soluble drugs that are commercially available, however, the success rates of nanoparticle formation was about 50%. It is considered that the key to the success is the intensity of intermolecular interactions between drug and additives. As the detailed mechanism is still not clear, it seems that the amorphous state of the drug might contribute to the formation and stability of the drug-loaded nanoparticles. Thus, it is important to select suitable additives for each drug. It is also not elucidated why the prepared particles have a homogeneous and spherical shape, but it is considered that the energy state of particles led to a thermodynamically more stable condition.

The high recovery rate of applied drug and the execution of this process under sterile conditions are considered to be the main advantages of this method. Therefore, it is considered to be suitable for treating a small amount but a wide variety of drugs. However, limited production rate is the biggest problem when considering its utilization for manufacturing pharmaceuticals. At present, this method can process 50 mg of drugs per hour. If this method is intended for use in the production of pharmaceuticals by pharmaceutical companies, the productive ability should reach at least the kg scale per hour. To improve the productivity, parallel running of laser irradiation or the further development of the laser itself would be necessary. This method is based on a new technology and we hope that it will be widely accepted in the pharmaceutical industry.

5. Conclusion

The results presented in this paper indicated that nanoparticle dispersion of poorly soluble drugs can be made by using new laser method. We indicated that homogenous CsA dispersion can be easily prepared without any degradation. As compared with ball milling method, we obtained CsA nanoparticles with more spherical shape and narrow size distribution. Furthermore, this technology can be used widely for the other poorly soluble drugs. The detailed mechanism of forming nanoparticles by laser irradiation has been the subject of our further studies.

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