초임계 유체기술을 이용한 나노 및 마이크로 입자의 약물전달시스템 제제의 설계

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Preparation of Nano- and Microparticlate Drug Delivery Systems Using Supercritical Fluid Technology

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Introduction

The pharmaceutical application of drugs is subject to a number of serious problems such as limited stability, low bioavailability, side effect, short half-time and enzymatic degradation in spite of the potential effect of medicine. In the pharmaceutical fields, over the past few decades, there has been achieved development of drug delivery dystems (DDS) that copes with these problems. Furthermore, drug carrier technology not only offers improvements of bioavailability, drug efficacy and toxicity but also doses a patient of low frequency [1,2]. The most important factor for designing DDS is to control the proper size and distribution of nano- and microparticulate DDS, which are generally composed of drug loaded microspheres, nanospheres or liposomes, such that their functions provide an access to the specific disease sites at right time through controlled release [3,4].

There are various techniques to fabricate drug delivery vehicles, such as organic phase separation, spray drying and solvent evaporation that are based on the use of excessive organic solvents. However, the major limits of these techniques lie in the fact that the irregular size distribution of microspheres and a large amount of remaining toxic solvents are frequently observed in the final products. Also, the use of high temperature and longer preparation time causes therapeutic peptides and proteins to be denatured, thus reducing their drug efficacy. To overcome these problems, the environmentally benign supercritical fluid technologies have been used as an alternative method to these traditional methods [5,6].

The supercritical state is obtained, as the temperature and the pressure of a substance increase above its critical values, where supercritical fluid shows liquid-like and gas-like properties simultaneously. Supercritical fluid has various thermodynamic and kinetic properties, suitable to the manufacturing of particulate DDS, such as high solubility, selectivity and compressibility, spontaneous separation by depressurization, low viscosity and surface tension, and high diffusion coefficient. Especially, carbon dioxide is most frequently employed for supercritical fluid technologies due to its relatively low critical temperature and pressure, non-toxicity, inexpensiveness, and non-flammability.

Based on the use of supercritical fluid as either solvent or non-solvent in the preparation of particulate DDS, supercritical fluid technologies are divided into two classes: The rapid expansion of supercritical solution (RESS) process, which uses supercritical fluid as a solvent, has the limitation that the drug to be loaded has to be dissolved in the supercritical fluid. Therefore, this technique is very suitable for the preparation of particulate DDS composed of nonpolar drugs and polymers soluble in supercritical carbon dioxide. However, therapeutic peptides, proteins and polar substances are in general poorly soluble in supercritical carbon dioxide and thus another approaches such as the supercritical antisolvent (SAS) process, the precipitation from compressed antisolvent (PCA) process

and the solution enhanced dispersion by supercritical fluids (SEDS) process are needed for the preparation of particulate DDS. In these processes polar drugs such as therapeutic peptides and proteins are first dissolved in a small amount of organic solvents and the solution is then continuously precipitated by spraying it through a nozzle and into the region of supercritical carbon dioxide acting as an antisolvent.

In the present study, the possibility of using the supercritical fluid technology for the preparation of particulate DDS composed of various different drugs such as therapeutic peptides, anticancer agents and hydrophobic antifungals and biodegradable polymers has been investigated.

Experimental

Materials

Three different drugs, leuprolide acetate, itraconazole, and 5-fluorouracil were obtained from Dong Kook Pharmaceutical Co. (Korea), Choong Wae Pharmaceutical Co. (Korea), and Sigma (USA), respectively. The biodegradable polymer, poly(L-lactide) (L-PLA) (MW: 40,000~70,000, i.v.: 0.80~1.20) was purchased from Polysciences Ltd. (USA). To prepare an inclusion complex of itraconazole, 2-hydroxypropyl-β-cyclodextrin (D.S.: 4.5) was purchased from Sigma (USA). To prepare particulate DDS, carbon dioxide (99.9 % purity, Dongmin Special Gases, Korea) was used as an antisolvent. All other reagents and solvents were of analytical grade, and used without further purification.

Preparation of Microspheres

A schematic diagram of the supercritical antisolvent apparatus used in this study is shown in Fig. 1. The precipitation vessel was pressurized with carbon dioxide using a high pressure pump at constant temperature. Using a syringe pump, steady-state conditions were achieved, and then the solution containing drug and polymer was sprayed into the vessel through a nozzle. Carbon dioxide was continuously fed into the vessel while maintaining a constant flow rate by controlling a metering valve. The microspheres precipitated were collected on a filter with an average pore size of 0.5 μm, placed at the bottom of the vessel. A depressurizing tank was equipped to recover organic solvent from the remaining solution.

Morphology

The morphology of the microspheres obtained was observed by using a scanning electron microscope (SEM) after coating the microsphere sample with gold-palladium on an aluminum stub.

Determination of Drug Content

Drug loaded microspheres were dissolved in methylene chloride and leuprolide was extracted into 0.1 M acetate buffer (pH: 4.0) by stirring the mixture for 1 hour. The buffer phase was separated by centrifugation and the leuprolide extracted was quantitatively determined by high performance liquid chlomatography (HPLC). Microspheres containing 5-fluorouracil were dissolved in methylene chloride and the drug was extracted into 0.1 M phosphate buffered saline (PBS, pH: 7.4). The content of 5-fluorouracil in the microspheres was assayed by using a UV-spectrophotometer.

In Vitro Drug Release

Approximately 20 mg of leuprolide acetate loaded microspheres were transferred to vials with 10 ml of 0.1 M PBS (pH: 7.4) and incubated at 37 ^{o}C and 100 stroke/min. According to a predetermined time schedule, the amount of leuprolide acetate released from the microspheres was determined by HPLC after filtering through a 0.5 μm filter. The amount of 5-fluorouracil released from the microspheres was quantitatively analyzed by using a UV-spectrophotometer. The release profile of itraconazole-2-hydroxypropyl-β-cyclodextrin inclusion complex was determined using the standard paddle method (USP XXIII). The dissolution medium was the 900 ml of pH 1.2 simulated gastric juice containing HCl and NaCl. Filtered samples were then assayed for itraconazole by HPLC.

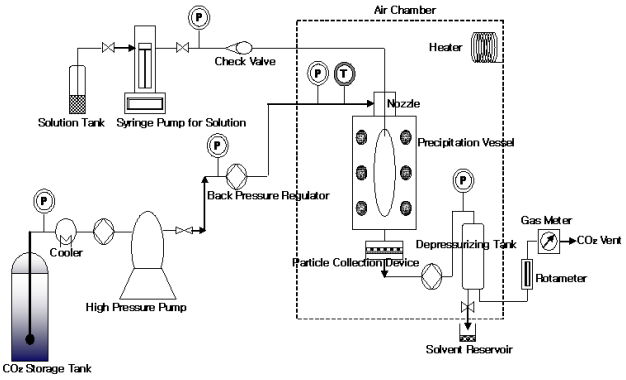
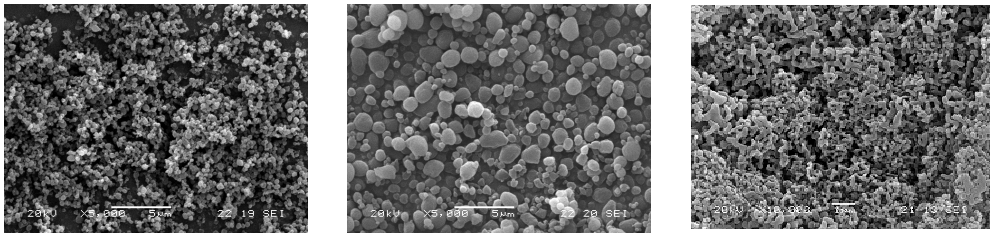


Fig. 1. Schematic diagram of the supercritical antisolvent(SAS) apparatus used for the preparation of microspheres.

Results and Discussion

The concentration of 5-fluorouracil or leuprolide in methanol was varied from 0.1 to 0.8 %(w/v) while the concentration of L-PLA in methylene chloride remaining constant to be 2.0 %(w/v). The operating pressure and temperature, flow rates of solution and CO_2 were maintained to be 13 MPa, 35 ^oC, 0.4 mL/min, and 20 L/min, respectively. Itraconazole and 2-hydroxypropyl-β-cyclodextrin were mixed in methylene chloride and ethanol and then sprayed into supercritical carbon dioxide at 35~65 ^oC and 8~32 MPa. The SEM micrographs of various drug-loaded microspheres prepared using supercritical fluid technology are shown in Fig. 2.



(A) (B) (C)

Fig. 2. SEM micrographs of various microspheres precipitated from supercritical carbon dioxide: (A) 5-fluorouracil-loaded L-PLA, (B) leuprolide-loaded L-PLA, (C) itraconazole-HP-β-CD inclusion complex.

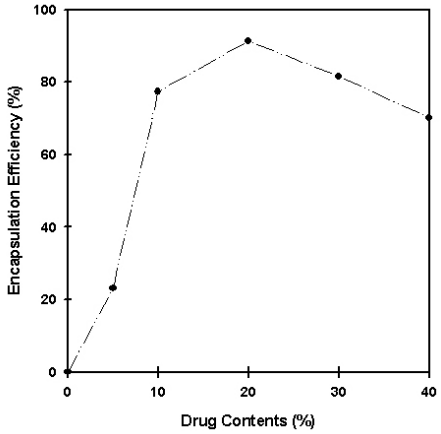
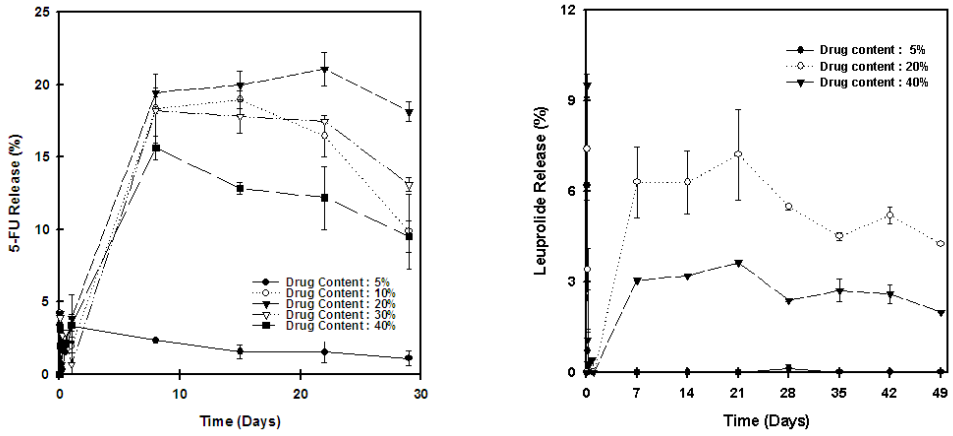


Fig. 3. Variation in the encapsulation efficiency as the weight ratio of 5-fluorouracil to L-PLA.

In the case of leuprolide and 5-fluorouracil, the mean particle size of the microspheres was not only

decreased but their morphology also became more agglomerated as the ratio of drug to polymer increased. The highest encapsulation efficiency was found to be 91 % for 5-fluorouracil loaded microspheres [Fig. 3]. Furthermore, the cumulative release profile was observed to be 95 % for 29 days when the ratio of 5-fluorouracil to L-PLA was 20 %. As shown in Fig. 4, the release of 5-fluorouracil and leuprolide from their microspheres was observed to be about 20 % and 5 %, respectively, for every 7 days.



(A) (B)

Fig. 4. *In vitro* release profiles of drugs from L-PLA microspheres in PBS at 37 °C: (A) 5-fluorouracil-loaded L-PLA, (B) leuprolide-loaded L-PLA.

Conclusion

Particulate drug delivery systems of 5-fluorouracil and leuprolide were fabricated with L-PLA for a prolonged release, and an inclusion complex of itraconazole was prepared for improved solubility and bioavailability by using supercritical carbon dioxide. The effective size reduction of particles was attained to micro- and nanospheres. Furthermore, the release profile was observed to follow the zero-order kinetics approximately.

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