Fabrication of controlled release devices for anticancer agents using supercritical antisolvent method

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Introduction

A major complication of most anticancer agents is the cytotoxicity effects on both cancerous and normal cells. Long term administration of the drugs to the cancer patient may result in some serious side effects such as hair loss, weight loss, and nausea. A delivery system that allows sustained release of drugs in the vicinity of target cells is desirable in the chemotherapy process to improve the overall quality of life of the patient. As a result, in the past few decades, much research has been carried out to develop micro-/ nanoparticles of biodegradable polymers as drug delivery systems for chemotherapy. We are interested in developing controlled delivery devices which give high yield of product and permit facile control of the product size and morphology through tuning of process parameters. In particular, the use of supercritical fluid techniques in the processing and fabrication of drugs and pharmaceutical products is examined in this research. This study focuses mainly on the Supercritical Antisolvent (SAS) [1-6] method of producing drug-loaded particles with high yield, controllable size and good morphology.

Several research groups have been working on the SAS process for semiconductor and catalytic materials, polymeric materials and drugs. Some studies have been performed on the application to controlled release devices, mainly for antibiotic and protein drugs. Few studies explore the *in vitro* release profile of the encapsulated polymeric devices [2-4]. In this work, we extend the application to the controlled release of paclitaxel. We investigate the effects of using various polymer blends to control the surface properties and the *in vitro* release profile of the drugs. The effects of addition of surfactants and emulsifiers to the organic phase were also explored.

Materials and methods

Materials

Poly (L-lactic acid) (PLA, Product Number P1566, MW = 85,000 – 160,000Da), Poly caprolactone (PCL, Product Number 181605, Typical MW = 65,000Da), Poly (DL-lactide-co-glycolide) (PLGA 85:15, Product Number 43,0471-1, MW = 50,000 – 75,000Da) and polyethylene glycol (PEG, Product number P4463, Typical MW = 8,000Da) were purchased from Sigma Aldrich. Compressed CO_2 was purchased from Soxal (Singapore Oxygen Air Liquide Pte Ltd). Vitamin E TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate) was purchased from Eastman Chemical Company. Paclitaxel was a generous gift from Bristol Myers Squibb. Dichloromethane (DCM, Product Number DS1432, HPLC/Spectro Grade) was purchased from Tedia (Tritech Scientific Pte Ltd, Singapore).

Microparticles and nanoparticles preparation

The experimental equipment for the SAS and modified SASEM processes is shown in Figure 1. Polymers and paclitaxel were first dissolved in DCM. The high pressure vessel, with a volume of 59 cm^3, was first filled with compressed CO_2. Liquefied CO_2 was subsequently pumped into the vessel by a high pressure pump to achieve the required pressure. The temperature in the vessel was controlled using a heated water bath. An ultrasonic vibration amplitude of 60 microns was used to create enhanced mixing of the organic – CO_2 phases. The organic solution was pumped into

the high pressure vessel at a flow rate of 2ml/min through a capillary tubing of ID 0.5 mm. After the batch precipitation process, the DCM-CO_2 mixture was vented off to a fume cupboard. Fresh CO_2 was introduced into the vessel at 50 bars for 3 times to remove any residual DCM in the particles. The particles were collected at the bottom of the vessel on a 0.22 micron cellulose acetate filter during the venting process

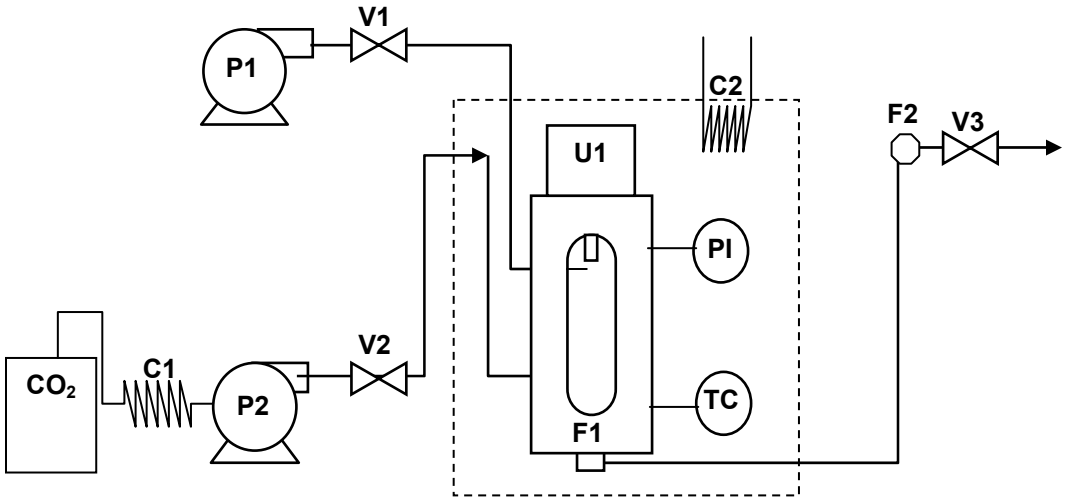


Figure 1. Experimental setup for modified SAS production of micro and nanoparticles. HP: Jerguson 12-T-32 high pressure vessel (For supercritical antisolvent process); U1: Ultrasonic system; Branson sonifier and converter, Sonics and Materials probe (3/8" probe tip diameter); C1: Polyscience 912 refrigerating circulator (for liquefying CO_2); C2: Polyscience 712 circulator with temperature control (Water bath); P1: Eldex B-100-S HP series pump (for solution injection); P2: Jasco HPLC pump (for pumping liquefied CO_2 into HP vessel); F1: 0.22 micron filter membrane (to collect particles from bottom of vessel); F2: stainless steel filter (0.22 micron, for filtering the contents leaving the vessel); V1: Swagelok 1/16" tube connection ball valve (solution line);V2: Swagelok 1/16" tube connection ball valve (CO_2 line); V3: Swagelok 1/8" tube connection ball valve (to vent); TC: Thermocouple connected to Thermometer read out; PI: Swagelok Pressure gauge.

Size and Surface morphology analysis

Qualitative observation of the size and surface morphology of the particles was achieved by scanning electron microscopy (SEM, JEOL JSM-5600 LV). Platinum coating (Autofine Coater, JEOL JFC-1300) of the samples was required before SEM analysis.

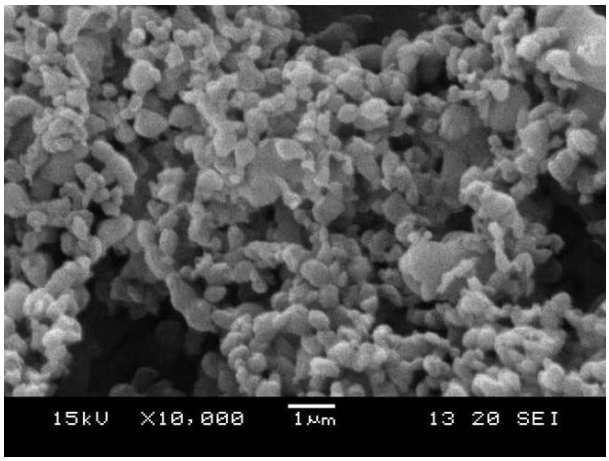
Thermogram properties analysis

Phase behavior of the particles was studied by differential scanning calorimetry (DSC, 2920 modulated, Universal V2.6D TA instruments). Approximately 2-10 mg of particles was loaded onto standard aluminum pans (40mg) with lids. The samples were

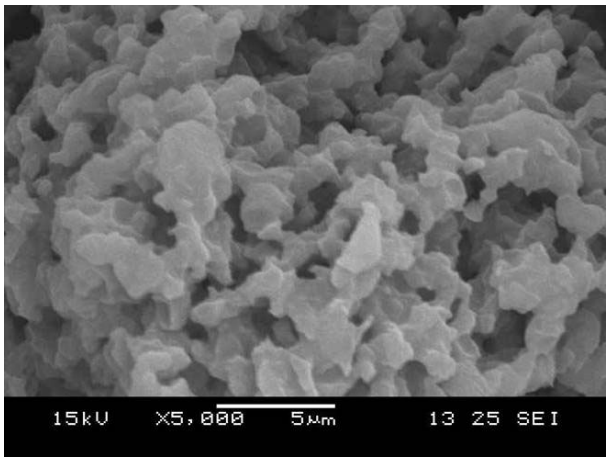
purged with pure dry nitrogen at flow rate of 5 ml/min. A blank aluminum pan was used as reference in all the experiments. The analysis was carried out using a temperature ramp of 10 Celsius/min from 20 to 250 Celsius.

Results and Discussion

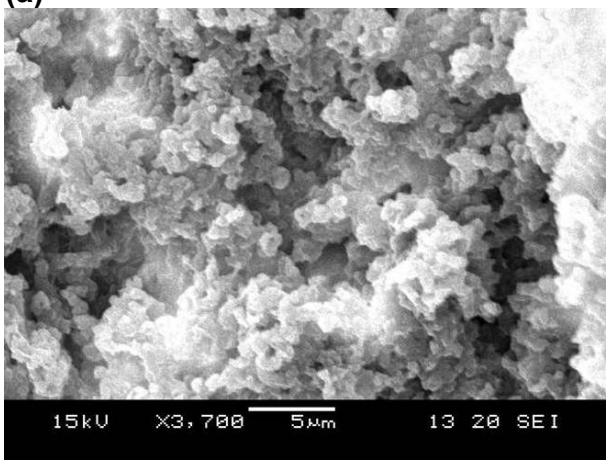
PLA, PCL and PLGA composites



(a)



(b)



(c)

Figure 2. SEM pictures of PLA and polymer blends at 2% polymer loading. (a) PLA only, 10,000 x magnification; (b) PLA/PCL 50:50w/w, 5,000 x magnification; (c) PLA/PLGA 50:50w/w, 3,700 x magnification.

The effects of co-precipitating PCL and PLGA with PLA were investigated. Different polymers have different degradation rates and structural matrices. By using mixtures of various polymers, we may alter the particle surface morphology and release profile of drugs from these particles. In this preliminary study, PCL and PLGA 85:15 were co-precipitated with PLA to examine the particle properties.

The size and surface morphology of the particles were analyzed by SEM. As shown in Figure 2, although powders were obtained for PLA, PLA/PCL and PLA/PLGA, only pure PLA yields discrete particles with minimal agglomeration. PLA/PCL and PLA/PLGA particles formed agglomerates which could not be suspended in deionized water.

Thermal analysis

The particles fabricated in this study were analyzed by differential scanning calorimetry (DSC). PLA and PCL have melting points at 173 – 178 ^{o}C [8] and 60 ^{o}C [8] respectively. PLGA 85:15 has a glass transition temperature around 50 – 55 ^{o}C [8]?

In previous studies with PLGA and PCL produced by the SAS process, no (or very few) particles were formed. Only polymer films could be obtained. In this study, it is of interest to study the co-precipitation of PLA with PCL and PLGA to determine if the powders obtained from the precipitation of the polymer blends were a mixture of the polymers, or simply PLA alone. DSC analysis was performed on the powder collected from the particles illustrated in Figure 2. Figure 3 shows the exotherm of the 3 samples. As shown in Figure 3, for blank PLA particles, there was only one endothermic peak at 175.8 ^{o}C, which corresponds to the endothermic melting point for PLA. Similarly, for samples PLA/PCL and PLA/PLGA, a distinct endothermic peak was also observed at 177.0 ^{o}C and 177.8 ^{o}C respectively. For PLA/PCL powder, there was an endothermic peak of similar amplitude at 59.5 ^{o}C (representing the melting point of PCL). The exotherm for PLA/PLGA also showed the characteristic glass transition onset at 51.28 ^{o}C. This showed that in the powder particle collected for co-precipitation PLA/PLGA and PLA/PCL, PLGA and PCL were present in the final product collected respectively. This showed that PLGA and PCL were able to precipitate in particle form in the presence of PLA.

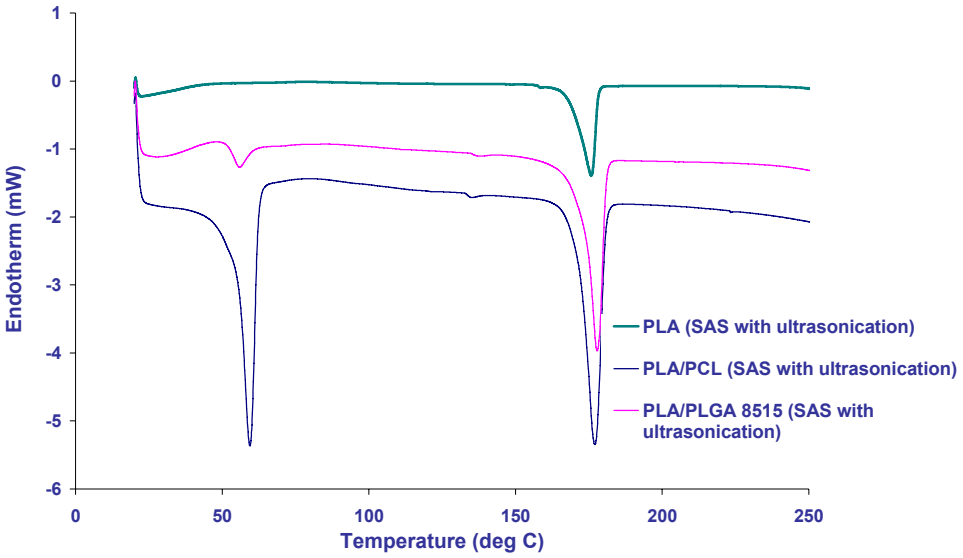
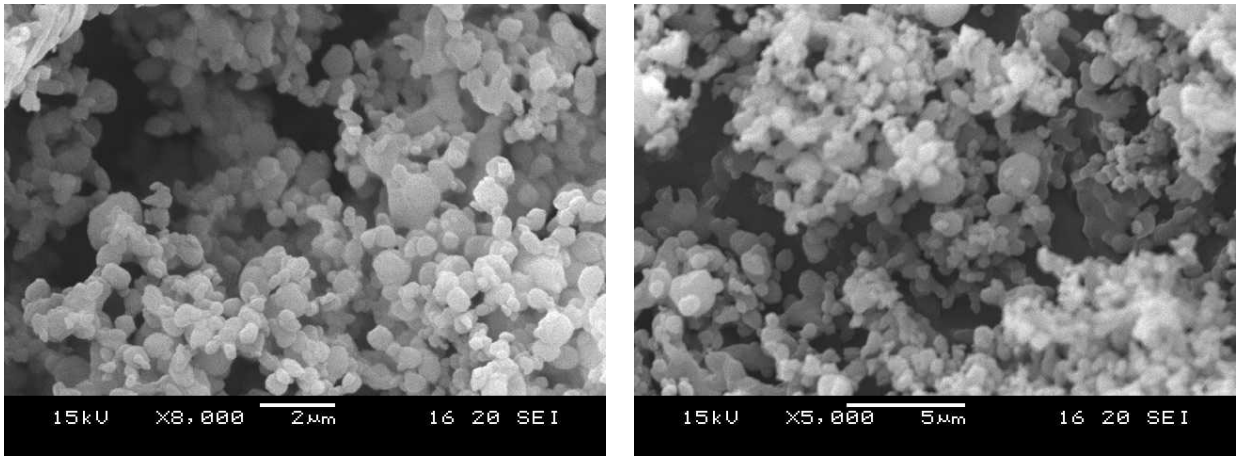


Figure 3. Thermogram properties for particles fabricated using modified SASEM.

Addition of various additives to PLA particles

The effect of various additives on the surface morphology and properties of the particles was also explored. These include experiments incorporating small amounts of PEG and vitamin E TPGS. Figures 4a and 4b below show the SEM pictures of PLA nanoparticles prepared using 5% PEG and 5% Vitamin E TPGS, respectively. Vitamin E TPGS can act as surfactant as well as matrix material. Addition of vitamin E TPGS does

not significantly improve the surface morphology of particles obtained in this study. PEG has excellent solubility in aqueous media but is non-biodegradable. However, it has low toxicity and can be readily excreted from the body via the kidneys. Addition of PEG seems to provide better particle morphology for the nanoparticles. Significantly less agglomeration was observed and the particle size was more uniform than for particles obtained without PEG.



(a) **(b)**

Figure 4. SEM pictures of PLA particles with additives. (a) with 5% PEG, 8,000 x magnification; (b) with 5% vitamin E TPGS, 5,000 x magnification.

Conclusion

Micro and nanoparticles of PLA, PLA/PLGA and PLA/PCL were fabricated in a modified SASEM process. With PLA, it was possible to obtain nanoparticles with a narrow size distribution. Due to the interaction of PLGA and PCL with supercritical CO_2, it was difficult to obtain powder-like particles. Additives were included in the PLA particles to examine the effects on particle size and shape. It was found that small amounts of PEG addition yield the best particle shape and size distribution. Further studies on the *in vitro* release profiles of the PEG-loaded particles will be carried out.

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