FORMULATION OF A CONTROLLED RELEASE CHEMOTHERAPEUTICAL SYSTEM BY THE ATOMIZED RAPID INJECTION SOLVENT EXTRACTION (ARISE) PROCESS

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ABSTRACT

Dense gas anti-solvent precipitation processes have been studied extensively over the past two decades for the development of novel drug formulations. Their advantages include tunable fluid properties and a reduced use of toxic organic solvents. The Atomized Rapid Injection Solvent Extraction (ARISE) process is a recently developed dense gas anti-solvent process. The use of capillary nozzles, required by most dense gas anti-solvent processes, is eliminated and process operation is greatly simplified. In this study, the feasibility of using the ARISE process for the co-precipitation of the hydrophilic anti-cancer drug 5-fluorouracil with poly L-lactic acid was demonstrated. The effects of the processing conditions on particle morphology, encapsulation efficiency and drug loading were studied.

1. INTRODUCTION

Using Dense Gas (DG) as an anti-solvent in particle engineering has shown success in producing nano- and microparticles with narrow size distribution[1]. Carbon dioxide (CO_2) is the most common DG used in pharmaceutical processing and has the ability to extract residual solvent from the product. An additional feature of DG processing is the intrinsically sterile processing environment[2]. Several configurations of DG anti-solvent micronization have been developed and the majority of them rely on the use of a capillary nozzle for solution atomization. An organic solution containing the compound to be micronized is atomized through a capillary nozzle at a constant flow rate and contacted with a CO_2 stream. Blockage of the nozzle is frequently encountered and scaling up the process involves sophisticated modeling and experimentation. The use of a low injection flow rate as required by the use of capillary orifices also limits the process output. The recently patented technique known as Atomized Rapid Injection Solvent Extraction (ARISE) was developed with the aim of overcoming some of these hurdles to make scale-up more economical and feasible. The use of a capillary nozzle has been eliminated. A solution with the dissolved solute is rapidly injected as a single bolus into a static supercritical fluid, energized by a gradient of pressure. Mixing of the solution and supercritical fluid is significantly intensified during the rapid injection. Variation of the solute concentration within the precipitation chamber is also minimized, leading to a more homogeneous precipitation. Another major advantage of the ARISE process is the reduction of the total number of control variables as atomization is independent of the

nozzle design (length, size and material), because there is none, and flow rates of solvent and anti-solvent. Process operation and optimization are significantly simplified.

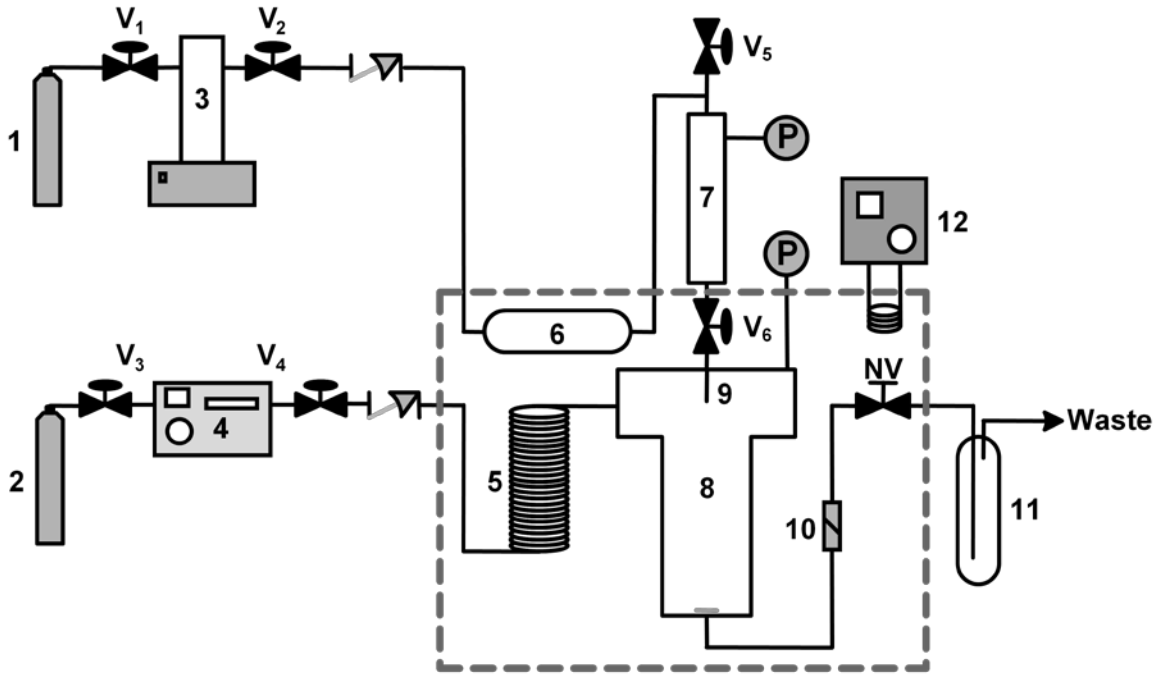
Previous studies on the ARISE process have demonstrated success in micronizing active pharmaceutical ingredients and exicipients[3]. However, no studies have been done on the co-precipitation of two compounds. In this study, the use of the ARISE process to co-precipitate the anti-cancer drug 5-fluorouracil with poly lactic acid has been evaluated. 5-Fluorouracil (5FU) is a popular chemotherapeutic agent for colon, breast, ovary, head and neck cancer. Despite its effectiveness in tumor suppression, it has several limitations. 5FU is a water-soluble drug and has a very short half-life (12 minutes) after intravenous injection in humans[4]. A controlled release formulation would greatly reduce the required dose therefore minimize the cytotoxicity effect. Poly lactic acid is a biodegradable polymer used in controlled release formulation which has been processed by DG anti-solvent systems. Previous study on the PLA-5FU system by conventional DG processing using a conventional anti-solvent precipitation method, namely the Solution Enhanced Dispersion by Supercritical fluids (SEDS) process was promising[5]. It is the aim of this study to investigate the feasibility of developing a controlled release formulation of 5FU with PLA using the ARISE process.

II. MATERIALS AND METHODS

The drug 5-fluorouracil (TCL grade) was purchased from Sigma. Poly (L-lactic acid) (MW 50,000) pellets were purchased from MP Biomedicals. Methanol (HPLC grade) and dichloromethane (HPLC grade) were purchased from Ajax Finechem Pty Ltd. and were used as received. Carbon dioxide ($\geq$ 99.5% purity) was purchased from Coregas Australia.

A schematic diagram of the experimental setup is presented in Figure 1. The precipitation chamber was first loaded with CO_2 at the desired pressure and temperature. The temperature and pressure of the CO_2 were maintained and controlled by the re-circulating heater and the reciprocating piston pump (Thar P-50) respectively. The precipitation vessel along with other critical equipment was immersed in a water bath at a constant temperature controlled by a recirculation heater (Thermoline Unistat). The injection chamber was loaded with the organic solution through V5. Nitrogen was pumped into the injection chamber and the back-pressure vessel to a specific back-pressure differential by a syringe pump (ISCO 250D). The organic solution was injected into the precipitation chamber by quickly opening V6 for 5 seconds. After the injection, the system was isolated for 10 minutes for stabilization. The rinsing process was subsequently started by passing fresh CO_2 at a continuous flow rate. The rinsing flow rate was controlled by adjusting the metering valve (V3). The precipitation vessel was dissembled for product collection after depressurisation of the precipitation chamber.

Experimental variables that were held constant are presented in Table 1. Co-precipitation of PLA and 5FU were performed at two temperatures (25^{o}C and 35^{o}C) in combination with the two polymer to drug ratios of 6:1 and 3:1. The solvent used in all the experiments was a 1:1 v/v dichloromethane: methanol (DCM:MeOH) mixture.



1. Nitrogen Supply
2. Carbon Dioxide Supply
3. Syringe Pump
4. Reciprocal Pump
5. Heating Coil
6. Back-pressure Vessel
7. Injection Chamber
8. Precipitation Vessel
9. 3.2mm OD Conduit
10. In-line Filter
11. Solvent Trap
12. Water Heater/Re-circulator

Lift-Check Valve

P Pressure Transducer

Water-Bath

V_n Ball Valve

NV Needle Valve

Figure 1 Schematic diagram of the ASES apparatus.

Table 1 Values of processing parameters held constant during process refinement.

Parameter	Value
Precipitation Vessel Volume (mL)	355
Back-pressure Differential (bar)	50
Volume of Injected Solution (mL)	10
CO_2 Rinse Mass Flow-rate (g/min)	10
CO_2 Rinse Mass (kg)	2.5

Particle morphology of the PLA-5FU composites was examined using scanning electron microscopy (SEM) (Hitachi S900). Total drug content in the product was measured by dissolving 5 mg of product in 2 mL of chloroform, followed by 6 mL of deionized water in a 10 mL centrifuge tube. The emulsion was shaken vigorously for 10 minutes to ensure complete extraction of 5FU into the water phase. The emulsion was then centrifuged for 30 minutes at 4800 rpm. The drug concentration of the extract (water phase) was determined by measuring the UV absorbance at 266 nm. The amount of unencapsulated drug was determined by gently rinsing 5 mg of product with 20 mL of methanol. The solution was filtered through a nylon syringe filter (0.45 μm) and the 5FU content was assessed by UV. The weight percentage of encapsulated drug was calculated by the difference between the total drug content and the unencapsulated drug content. Drug loading, encapsulation efficiency and total drug recovery were calculated according to the following equations. The encapsulation efficiency in this study was calculated in a way that is comprehensive of the process yield instead of merely comparing final and initial polymer to drug composition. Total drug recovery was also reported in this study.

$$Drug\ Loading\ (wt\%) = \frac{mass\ of\ encapsulated\ 5FU}{Mass\ of\ PLA + Mass\ of\ encapsulated\ 5FU} \times 100\%$$

$$Encapsulation\ Efficiency\ (\%) = \frac{mass\ of\ encapsulated\ 5FU}{Mass\ of\ 5FU\ in\ the\ starting\ organic\ solution} \times 100\%$$

$$Total\ Drug\ Recovery\ (\%) = \frac{total\ mass\ of\ 5FU\ in\ the\ product}{mass\ of\ FU\ in\ the\ starting\ organic\ solution} \times 100\%$$

III. RESULTS AND DISCUSSIONS

Particle Morphology

Scanning electron microscopy images of the PLA-5FU microparticles are presented in Figure 2 and Figure 3. Coprecipitation of PLA and 5FU by ARISE was successful with spherical submicron particles obtained from all the experimental conditions. Two particle populations were observed. Based on previous studies on the precipitation of neat 5FU and neat PLA, the smaller particles (~500nm) are identified as 5FU and the larger particles (~1μm) as PLA-5FU. The polymer particles obtained at 35^{o}C are slightly larger than at 25^{o}C for both polymer:drug ratios. One possible reason could be the more significant lowering effect of the supercritical fluid on the glass transition temperature of the polymer, hence larger PLA particles are produced. The same observation is reported in the literature[6]. Overall, the effects of temperature and polymer to drug ratio on particle morphology were negligible.

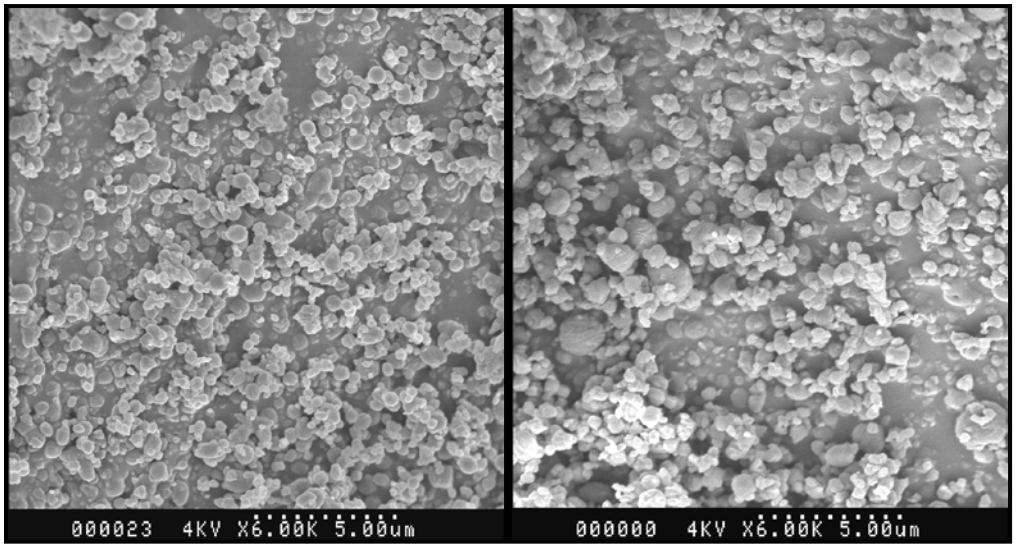


Figure 3 PLA-5FU microparticles produced by ARISE at 90 bar, PLA:5FU ratio of 6:1; precipitation temperature (left) 25°C, (right) 35°C bar.

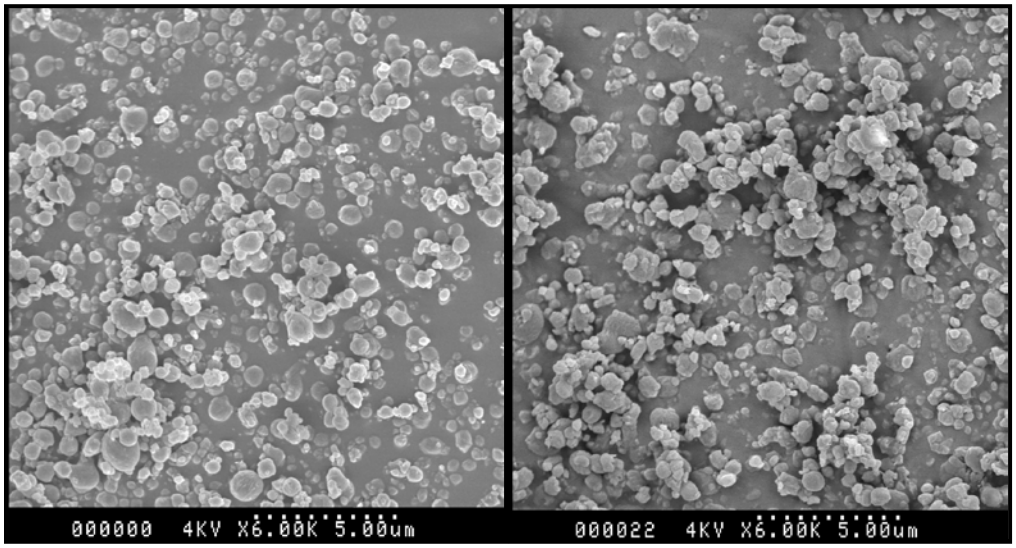


Figure 4 PLA-5FU microparticles produced by ARISE at 90 bar, PLA:5FU ratio of 3:1; precipitation temperature (left) 25°C, (right) 35°C bar.

Drug Loading and Encapsulation Efficiency

Product and process characterization are summarized in Table 2. Polymer concentration was kept constant at 12 mg/mL for all experiments. At a polymer:drug ratio of 6:1, the increase in processing temperature from 25°C to 35°C resulted in an almost two-fold increase in drug loading and encapsulation efficiency. The increase in temperature may enhance the solvent extraction resulting in a faster precipitation of the drug. At the lower polymer:drug ratio of 3:1, the effect was not observed. One possible reason is that the concentration of 5FU (4mg/mL) is very close to the saturation concentration in the 1:1 DCM:MeOH mixture. Precipitation rate would be faster than in the 6:1 ratio experiments due to a higher degree of supersaturation of the drug. The high degree of supersaturation of the drug may have dominated the effect of more efficient solvent extraction at high temperature. Therefore drug loading for S3 and S4 are higher than S1 and S2, respectively,

but are similar among the two temperatures studied. The slightly lower drug loading of S4 compared to S3 was likely due to the enhanced solubility of 5FU in the methanol:CO_2 system at higher temperature which was dominating the higher solvent extraction rate. The decrease in total drug recovery from 93.24% to 85.62% supports this hypothesis. It should be noted that DG anti-solvent precipitation is a highly complex process with several interacting and competing effects and therefore it is almost impossible to isolate the effect of one parameter.

Table 2 Summary of process and product characterization of the PLA/5FU composites. Experimental conditions: pressure: 90 bar; polymer concentration: 12 mg/mL; solvent: DCM:MeOH 1:1 v/v.

Sample	Process Condition	Initial Solution	Process and Product Characterization		
Code	T [^oC]	Polymer:Drug Ratio	Total Drug Recovery [%]	Drug Loading [wt.%]	Encapsulation Efficiency [%]
S1	25	6:1	79.99	1.65	8.29
S2	35	6:1	90.47	3.06	15.47
S3	25	3:1	93.24	6.81	17.98
S4	35	3:1	85.62	6.40	15.94

Drug loading and encapsulation efficiency were comparable with the reported values (3.05 wt%, 17.8%) for conventional SEDS at 33^{o}C, and 120 bar[5]. Drug loading and encapsulation efficiency obtained from this study are encouraging, considering the fact that the process has not been optimized. Base on these preliminary experiments, it is expected that improvement in the drug loading and encapsulation efficiency would be possible by further investigation of the effect of the precipitation pressure, polymer to drug ratio and the solvent composition.

IV. CONCLUSIONS

Submicron particles of PLA and 5FU composites were successfully produced by the ARISE process. Within the range of conditions investigated, there was no significant effect of temperature and polymer:drug ratio on the particle morphology. The highest drug loading and encapsulation were 6.81wt% and 17.98% respectively at 25^{o}C, 90 bar and 3:1 polymer: drug ratio. The PLA-5FU microparticles can be potentially employed as a controlled release formulation for cancer therapy. Further studies are required to optimize the drug loading, encapsulation efficiency and release kinetics.

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