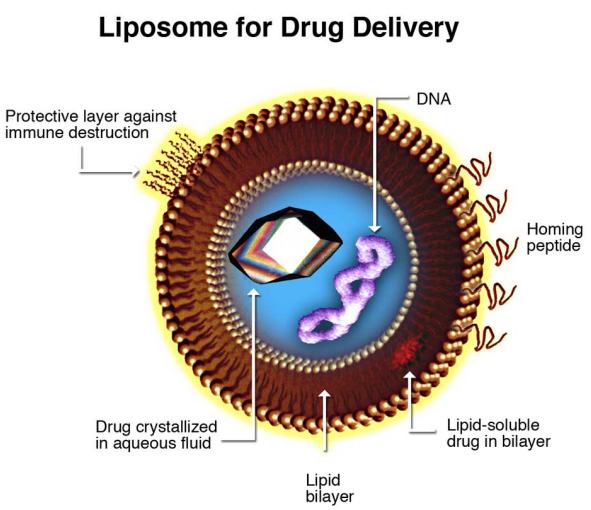
Preparation of Proliposomes using Supercritical Fluid

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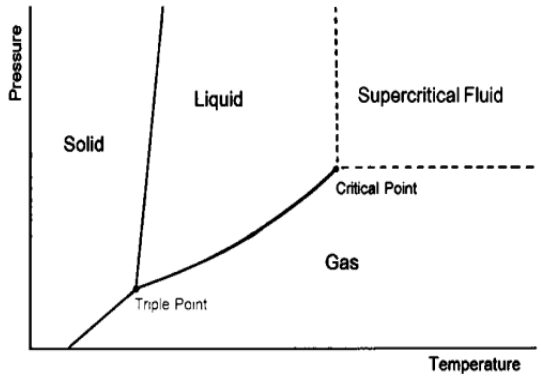


Figure 1: Phase diagram of CO2.[1]

Background: Liposomes are being widely used today as they can easily transfer drugs across membranes.[2,3,4] However, liposomes have a short shelf-life. Proliposomes are developed to overcome these limitations. They are solid particles composed of phospholipids and drug molecules that transform into liposomes upon hydration prior to administration.[5] Several conventional methods are used for the preparation of proliposomes. These methods, however, use a considerable amount of organic solvents which impact the environment. Supercritical fluid (SCF) technology have been introduced to overcome these challenges. This technology offers a one-step process, while operating at mild temperatures, making it suitable for thermal-labile compounds like anticancer drugs. SCFs properties, such as density, diffusivity and viscosity, can be controlled by simply altering the temperature and pressure during operation. The methods selected depends on the selection of SCF, SCF unit processing limits, and physico-chemical properties of the drug molecules and excipients.[6]

Aim: To produce proliposomes using supercritical carbon dioxide.

Materials: 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) with a purity of >99% was kindly donated by Harry Parekh's group (PACE research labs, UQ). Carbon dioxide was purchased from BOC. Normal saline (0.9% NaCl) was purchased from Chem-Supply (UQ) and absolute ethanol was purchased from Sigma-Aldrich.

Method: DSPC of 2mg was put in a mixer and the supercritical carbon dioxide ($scCO_2$) was introduced to saturate the solution. The stirrer was set at 155 rpm and the parameters were varied according to the design constructed as provided in Table 1. The solution was then expanded once the system had been slowly depressurized to atmospheric conditions through a nozzle. This sudden drop of pressure led to the formation of gas CO_2 from $scCO_2$ which was then released back into the air.
Atomization was accomplished to produce proliposomes. A flow diagram of the experimental design is shown in Figure 2. The proliposomes obtained were mixed with 1mL of normal saline for hydration to form liposomes. The suspension was then mixed well using a rotary evaporator for 15 minutes in a water bath at 65°C.

Table 1: Experimental domain

Factors	-1	0	+1
Processing time	10 minutes	30 minutes	60 minutes
Temperature	25°C	40°C	50°C
Pressure	60 bar	150 bar	250 bar

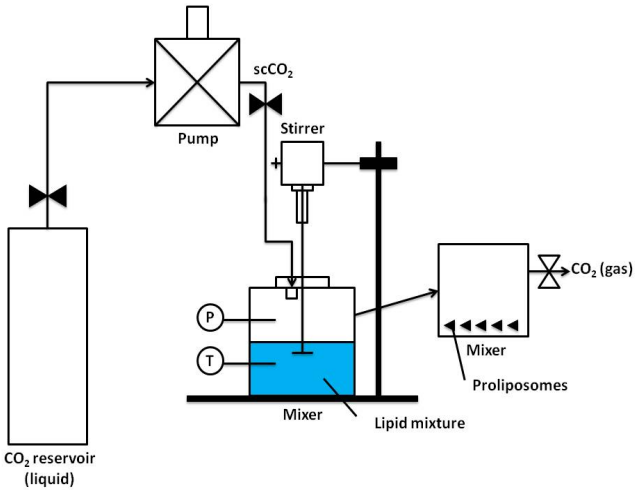


Figure 2: Flow diagram of the supercritical fluid equipment used at PACE/UQ.

Discussion: The yield of the liposomes produced were not significantly affected by temperature, pressure and processing time. The reason to this is because the lipid suspension and hence, the product remained in the mixer throughout the experiment. $ScCO_2$ in this study behaved as a solvent. The results obtained shows that the liposomal size was the only effect that was significantly influenced by the three factors used in this study. Thus, it can be deduced that the size of the liposomes was smallest when the factors were at their maximum. However, temperature could have been confounding with pressure and processing time which may have affected the response curve. This can be explained by the density of $scCO_2$ which is highly influenced by temperature and pressure.

Conclusion: This study demonstrated that proliposomes were successfully produced using SCF technology while eliminating the use of organic solvents. It can be observed that pressure, temperature and processing time significantly influenced the size of liposomes, proving that a higher pressure, temperature and processing time produced smaller liposomal size.

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