SUPERCRITICAL IMPREGNATION OF ANTICANCER DRUG INTO BIODEGRADABLE POLYMER

Keisuke Sato[1], Satoshi Yoda[2], Hideko T. Oyama[1],
1 Department of Science, Rikkyo Univ., Tokyo, Japan,
2 National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

Introduction

Poly (lactic acid) (PLA) is a useful biodegradable polymer with biodegradability, biocompatibility and bioresorbability. PLA is thus employed as a variety of medical materials including controlled release drug delivery system (DDS). There are two major routes on impregnating drug into PLA matrix; one is immersion using an organic solvent and the other is mechanical mixing. However remaining harmful solvent in the PLA matrix or deterioration of drugs processed at elevated temperature on mixing have been problems on DDS material fabrication.

Recently an alternative route using supercritical carbon dioxide ($scCO_2$) as a media for drug impregnation has been developed. PLA matrix is immersed in the $scCO_2$ solution of drugs under high pressure. $scCO_2$ is an environmental friendly, non-flammable, and non-toxic solvent and is evaporated from the PLA matrix after use. $scCO_2$ is capable of plasticizing and swelling PLA at lower temperature than that in atmosphere, and it is helpful to prevent drugs from deterioration. Thus the $scCO_2$ route is quite beneficial for preparation of DDS materials.

Solubility of drugs into $scCO_2$ is one of the most important factors for successful impregnation process. However, solubility of drugs, which generally have a large molecular weight and /or molecular polarity, is quite low. It is well known that addition of polar solvents such as ethanol can increase solubility of many polar substances into $scCO_2$. However, mainly due to difficulty of measurements, there are limited data of drug solubility in the $scCO_2$-organic solvent mixture. Additionally, there are few literature on PLA swelling or plasticization in the mixture.

We have developed the novel fabrication route of PLA-based DDS material using ethanol (EtOH) and CO_2 mixture. EtOH is believed to be the only one organic solvent acceptable to preparation of medical materials. Here we demonstrate impregnation of an anticancer drug, paclitaxel (PT) into amorphous poly ($_{DL}$-lactic acid) ($P_{DL}LA$) in the EtOH-CO_2 mixture at 313 K and 20 MPa. Solubility measurement of PT and observation of the $P_{DL}LA$ swelling in the EtOH-CO_2 mixture were performed. The solubility of PT to $scCO_2$ have already reported and that to EtOH-CO_2 mixture was mentioned in a literature[1] but the data region is limited. $P_{DL}LA$ swelling in $scCO_2$ was reported in a few researches[2][3] but no literature on the swelling in the EtOH-CO_2 mixture. The efficacy of the mixture will be discussed in detail.

Experimental

Materials

The paclitaxel (PT) (purity 99.74 %, Tokyo supply, Co. Ltd) was used without further purification. The $P_{DL}LA$ used in this work (Mitsui Chemicals, Inc.) contains approximately 12 % D-lactide, which has the number average molecular weight (M_n) and the weight average molecular weight (M_w) of 117,000 and 157,000, respectively. This $P_{DL}LA$ denoted as PLA117k in the present work was molded to an adequate shape first by a hot-press at 463 K, 5 min at 10 MPa and then by a cold-press at 273 K. CO_2 gas (99.99%) was dehydrated using a zeolite column and used for all experiments. Conventional EtOH (99.9%) and acetonitrile (analytical grade) for HPLC were used without further purification.

Solubility measurement of PT in EtOH-CO₂ mixture

Solubility of PT in EtOH-CO_2 mixture was measured by direct injection of the solvent into high performance liquid chromatography (HPLC). The details of measurement using HPLC has been described in our previous publication.[4] Schematic diagram of the high pressure flow system is shown in Fig.1. A molar composition of EtOH and CO_2 were controlled by flow rates of pumps (2) and (3). EtOH and CO_2 were mixed in the accumulator (4) and adjusted at 313 K and 10-37 MPa by a thermo static bath (7) and a backpressure regulator (9). PT was filled up into the sample cell (5) with glass beads (0.7-1 mm in diameter). PT was then dissolved to be saturated in the EtOH-CO_2 mixture. The flow rate was carefully adjusted to keep the dissolution equilibrium.

① CO_2 cylinder
② CO_2 high pressure pump (cooled at 273 K)
③ EtOH high pressure pump
④ Accumulator
⑤ Sample cell
⑥ Sampling valve (2 μl)
⑦ Thermo static bath
⑧ Pressure gauge
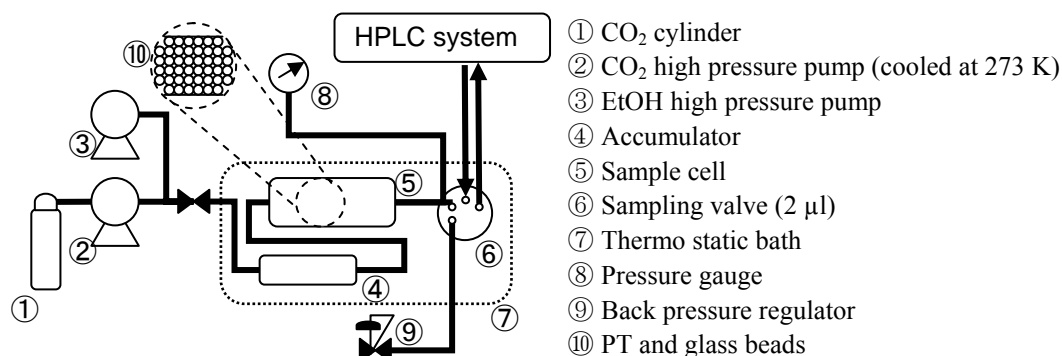⑨ Back pressure regulator
⑩ PT and glass beads

Fig.1 Solubility measurement apparatus

The HPLC analysis involved acetonitrile-water (50: 50 (v/v)) mixture as eluent and a column (MetaChem taxsile, 250 mm (L) x 4.6 mm (ID), VARIAN, Inc.). The absorption of PT at 230 nm was monitored via UV-VIS detector. The PT is detected in HPLC after the EtOH and CO_2 are dissolved in the HPLC eluent. In other words, molar composition of EtOH and CO_2 and the mixture's pressure do not directly affect the PT analysis. A calibration curve of PT dissolved in the acetonitrile-water mixture was established before the analysis. The mole fraction solubility of PT in the mixture, S_{PT} was determined using the following equation:

$$S_{PT} = \frac{n_{PT}}{n_{PT} + n_{CO2} + n_{EtOH}} \qquad \text{(i)}$$

where n_{PT}, n_{CO2} and n_{EtOH} are the number of moles in the sampling valve(2 μl) of PT, CO_2 and EtOH, respectively. n_{PT} was calculated from the calibration curve and the volume of the valve. Due to the lack of established density data of EtOH-CO_2 mixture, n_{CO2} and n_{EtOH} are defined as follows:

$$n_{CO2}=(\rho_{CO2}/M_{CO2})\,[V_{valve}\,\{FR_{CO2}/(FR_{CO2}+FR_{EtOH})\}] \qquad (ii)$$

$$n_{EtOH}=(\rho_{EtOH}/M_{EtOH})\,[V_{valve}\,\{FR_{EtOH}/(FR_{CO2}+FR_{EtOH})\}] \qquad (iii)$$

where ρ_{CO2} is CO_2 density at the measurement temperature and pressure, ρ_{EtOH} is EtOH density at 293 K, M_{CO2} and M_{EtOH} are the molecular weight of CO_2 and EtOH, FR_{CO2} and FR_{EtOH} are the flow rate of pumps (2) and (3), and V_{valve} is the volume of sampling valve(2 μl). The ρ_{CO2} was calculated from the equation of state developed by Span and Wagner[5]. EtOH density at atmospheric pressure was employed here due to the lack of established density data around the experimental condition. Then the solubility of PT is not high ($S_{PT}< 10^{-4}$), we assumed that the volume of PT in the sampling valve was negligible.

Swelling observation of PLA117k in EtOH-CO₂ mixture

PLA117k swelling in EtOH-scCO₂ mixture was observed at 313 K and 6-20 MPa by direct visualization using a high pressure view cell. Fig. 2 shows a schematic structure of the view cell (7). The $P_{DL}LA$ disk (12) (6 mm in diameter and 0.6 mm in the thickness) was fixed between sapphire windows (10) with c-style spacer ring (11) (0.5 mm in thickness). The cell was maintained at 313 K during the experiments. After evacuating the sample cell at least for 24 h, the EtOH-CO₂ mixtures (0-100 mol % EtOH) at 313 K and 6-20 MPa was prepared using pumps (2 and 3) and introduced in the cell (7). Homogenous phase conditions of the EtOH-CO₂ mixture[6] was selected to simplify the experiment.

① CO₂ cylinder
② CO₂ high pressure pump (cooled at 278 K)
③ EtOH high pressure pump
④ Accumulator
⑤ Buffer tank
⑥ Pressure gauge
⑦ High pressure view cell with cartridge heater
⑧ Video camera
⑨ Back pressure regulator
⑩ Sapphire glass (view window)
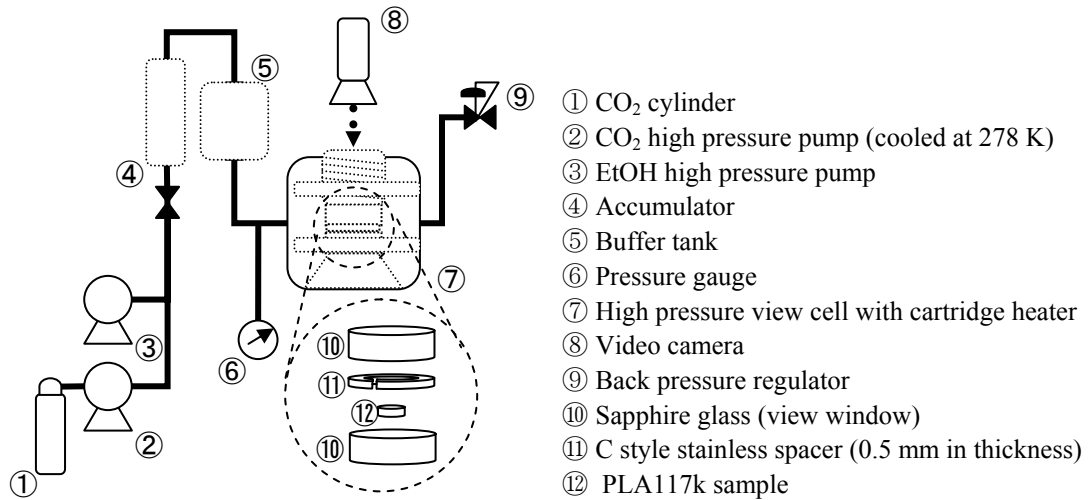⑪ C style stainless spacer (0.5 mm in thickness)
⑫ PLA117k sample

Fig.2 Direct visualization of polymer swelling apparatus

The appearance, the size and the area of PLA117k were recorded by video camera (8). Since the PLA117k is fixed between the windows to have a constant gap, the vertical swelling can be neglected. Then the swelling degree (s) is defined as the following equation;

$$s = \frac{A-A_0}{A_0} \qquad (iv)$$

where A_0 and A are areas of PLA117k before and after the swelling experiments, respectively.

Impregnation of PT in PLA117k

Apparatus for the impregnation experiments is shown in Fig.3. Ten mg of PT and the PLA117k disk (5 mm in diameter and 1 mm in thickness) were placed separately in a high pressure cell (5). The EtOH-CO_2 mixture (0-100 mol% EtOH) at 313 K and 20 MPa was then introduced into the cell and maintained the condition for 24 h without flowing. After cooling the cell to room temperature, the EtOH-CO_2 was slowly released using the back pressure regulator (8). The PT impregnated sample was evacuated to remove ethanol and used for PT content analysis.

① CO_2 cylinder
② CO_2 high pressure pump (cooled at 273 K)
③ EtOH high pressure pump
④ Accumulator
⑤ High pressure cell
⑥ Thermostatic bath
⑦ Pressure gauge
⑧ Back pressure regulator
⑨ Glass plate
⑩ Stainless mesh
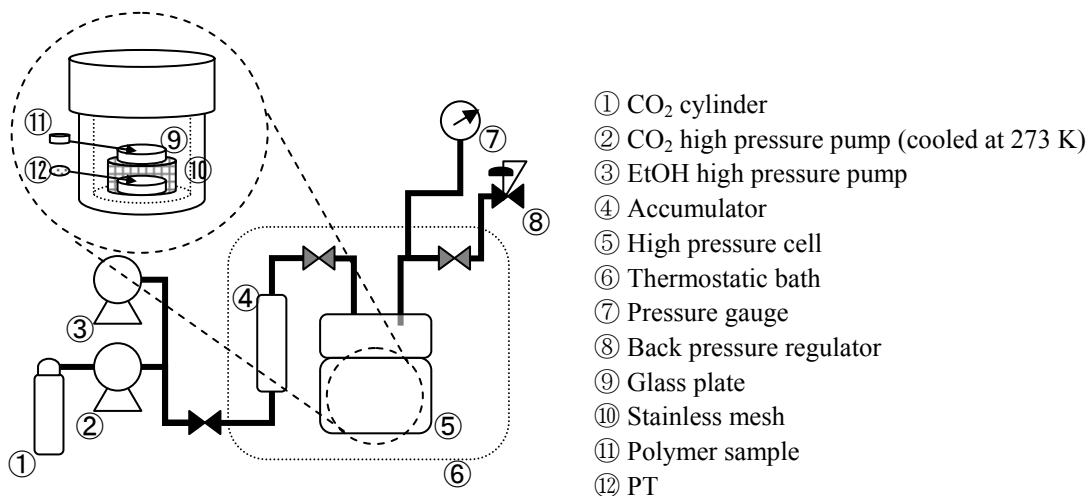⑪ Polymer sample
⑫ PT

Fig.3 Impregnation of PT into PLA117k apparatus

The amount of impregnated PT in the PLA117k disks were determined using gel permeation chromatography (GPC). The sample was dissolved into chloroform. The GPC analysis was conducted using (TSK-GEL, 300 mm (L) x 7.8 mm (ID), Tosoh corp.) and chloroform as eluent at (313 K). The PT and PLA117k can be detected separately at this condition. Both UV-VIS and fluorescence detectors were employed for PT detection. The UV-VIS absorbance of PT was measured at 250 nm. The fluorescence intensity of PT was monitored at 310 nm with the excitation at 250 nm.

Results and discussions

Solubility of PT in EtOH-CO_2 mixture

Solubility of PT in $scCO_2$ and EtOH-CO_2 mixture was shown in Fig. 4. The solubility of PT in $scCO_2$ located between $S_{PT}=1.2$ to 2.1×10^{-6}. Results were in accordance with those in literatures [7-8]. Solubility of PT in 10 mol% EtOH-90 mol% CO_2 mixture was $S_{PT}=1.5$-1.9×10^{-5} at 313 K, 10-15 MPa, which is 9.5-13 times larger than that in the pure $scCO_2$. Since the solubility of PT in EtOH is $S_{PT}=1 \times 10^{-3}$, the solubility in the mixture is expected to increase with enhancement of the EtOH content. Solubility in the mixture with larger EtOH content is under investigation.
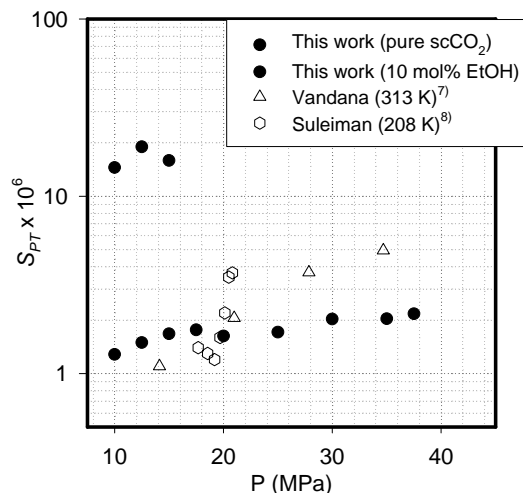
Fig.4 Solubility of PT in the scCO$_2$ and EtOH-CO$_2$ mixture

Swelling of PLA117k in EtOH-CO$_2$ system

Fig.5 shows profiles of PLA117k swelling in a variety of EtOH-CO$_2$ mixture at 313 K. Larger PLA117k swelling was observed in EtOH-CO$_2$ mixture than that in scCO$_2$. No swelling of PLA117k was observed in EtOH, like crystalline Poly (L-lactic acid) (P$_L$LA) as mentioned in the literature[9]. The mixture with 25-50 mol% EtOH induced substantial swelling especially at high pressure. Within our experiments, the maximum swelling was observed in the mixture with 40 mol% EtOH at 20 MPa. It is noteworthy that appropriate EtOH-CO$_2$ mixture was observed to conduce to significant PLA117k swelling, which is larger than that in scCO$_2$.
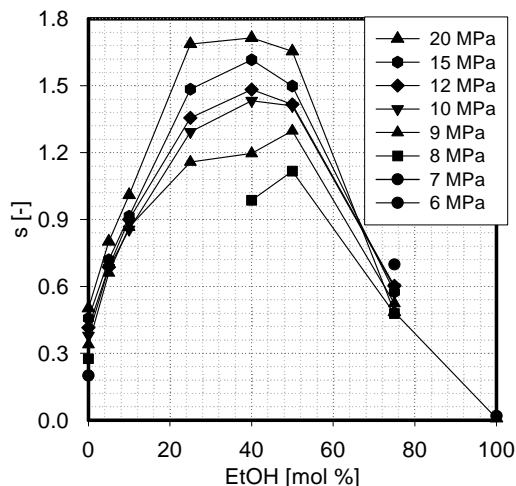

Fig.5 Swelling of PLA117k in EtOH-CO$_2$ mixture

Impregnation of PT in PLA117k via EtOH-CO$_2$ systems

Impregnations of PT into PLA117k at 313 K, 20 MPa with a variety of EtOH-CO$_2$ mixtures (0-100 mol% EtOH) were investigated. The amount of impregnated PT in pure scCO$_2$ was 0.1 μg/ mg (impregnated PT/ PLA), whereas that in EtOH-scCO$_2$ mixtures were 4-10 times larger than that in pure scCO$_2$ (0.4–1.1 μg/ mg). Little amount of PT was impregnated into PLA117k in EtOH solution.
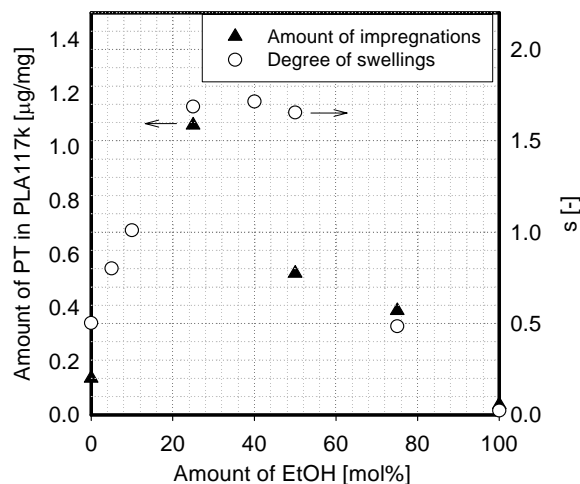
Fig.6 Amount of impregnations and degree of swellings in the mixtures

Fig.6 shows the relationship between the EtOH molar ratio in EtOH-CO_2 mixture at 20 MPa, 313 K and the amount of impregnated PT. The maximum swelling at the same condition was also shown for comparison. The results of PT content are approximately correlated with results of swelling. Even though PT solubility to the mixture expected to be increase significantly with EtOH, the amount of PT inversely decreased with EtOH content. It is presumed that diffusion of impregnating media in PLA117k matrix plays important role in the system. The use of EtOH-CO_2 mixture is more beneficial than the scCO$_2$ system due to larger PT solubility and swelling, and than the EtOH system having no swelling effects.

The maximum of PT amount was not in accordance with the maximum of swelling. We consider the effects of depressurization step. A part of impregnated PT in the mixture under a high pressure could be removed from the PLA117k matrix during the depressurization process. This process would result in complicated change in both solubility and swelling. Further research on depressurization process should be conducted.

Conclusion

Impregnations of PT into PLA117k in various compositions of the EtOH-CO_2 mixture were investigated at 313 K and 20 MPa. It was proved to be more advantageous to use the EtOH-CO_2 mixture for impregnation of PT than the pure scCO$_2$. The amount of impregnated PT in EtOH-scCO$_2$ mixtures (0.39–1.08 μg/ mg) was 3-8 times larger than that in pure scCO$_2$ (0.14 μg/ mg), and was 10-27 times than that in EtOH (0.04 μg/ mg). The EtOH-CO_2 mixture with 25 mol% EtOH showed the maximum amount of impregnated PT in PLA117k. The increase in the amount of impregnated PT was probably attributed to the solubility of PT into the EtOH-CO_2 mixture and the swelling of PLA117k. The solubility of PT in the mixture with 10 mol% EtOH was 13 times higher than that in scCO$_2$ at 313 K and 15 MPa. The maximum swelling of PLA117k in the mixture containing 40 mol% EtOH at 313 K and 20 MPa was observed as 1.7 times larger than the initial size. Therefore, it was demonstrated that EtOH-CO_2 system is promising for fabrication of polymer-based DDS materials.

References

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