

Distribution of Pores in PLGA_{85:15} and PLGA_{50:50} Foams Manufactured by the scCO₂ Process

Arfan Subhani^{a,*}, Selmi Erim Bozbag^{b,d}, Veronique Santran^c, Jean-Stephane Condoret^b, Severine Camy^b and Alain Lamure^a

^a CIRIMAT/INP – 118 Route de Narbonne, 31077 Toulouse Cedex 04,

^b Laboratoire de Génie Chimique, BP 1301,5 Rue Paulin Talabot, 31106 Toulouse Cedex 1

^c ICELLTIS, Parc Technologies Cap Delta, Delta sud, 09340 Verniolle

^d Dept. of Chemical & Biological Engineering, Koc University, Sariyer 34450, Istanbul Turkey

Abstract:

Scaffold preparation in tissue engineering is advancing by using various biodegradable polymers such as Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), and the co-polymer, known as Poly(lactic-co-glycolic acid) (PLGA). Porous structures of the biodegradable Poly (lactic-co-glycolic acid) (PLGA_{50:50} and PLGA_{85:15}) were prepared with the batch foaming technique (pressure quench) using supercritical CO₂ as blowing agent. The effect of one of the process parameter depressurization rate (dP/dt) on the final porous structure was investigated. A deep study proved that the diffusion of CO₂ into the polymer, the solubility of CO₂ in the polymer, the depression of the glass transition temperature of the polymer and the nucleation are the affecting phenomena. It was found that the pore size is decreasing with the increasing rate of depressurization. The very close porosity values obtained at 5 bar/s and 10 bar/s can also be explained by the coalescence of the pores during desorption of the CO₂. Our observations on pore size and the porosity (the swelling) of the polymer have shown that with great pore sizes comes great porosity and with small pore size comes small porosity. This behavior is in agreement with the literature [1]. By our knowledge, it is impossible to create big pores with small polymer volume or small pores with important polymer swelling. However the interconnectivity in the scaffold was not of very high quality.

KEY WORDS:

Poly(lactic Copolymers, Foams, Supercritical CO₂, Design of Experiment, Pores Distribution.

1. Introduction

Different biodegradable scaffolds are described in the literature. Most of these scaffolds come from the family of polyesters. Poly (α -hydroxy acids) like Poly (lactic acid) (PLA), Poly (glycolic acid) (PGA), and the co-polymer, known as Poly (lactic-co-glycolic acid) (PLGA) are a part of the tissue engineering studies. The common use of these polymers is basically related to their degradation behavior. PLA degrades into lactic acid, and PLGA degrades into lactic and glycolic acid. Also, for PLGA, the degradability rate can be controlled by changing the co-monomer composition. Furthermore, PLA and PLGA are approved by United States Food and Drug Administration for biomedical uses [2]. In some cases, scCO₂ was applied for the foaming of biodegradable polymers such as poly (lactic-co-glycolic acid) [3, 4], poly (3-caprolactone) [5, 6, 7] and poly(lactic acid) [8].

Different techniques at different ranges are proposed to manufacture scaffolds. Fiber bonding, solvent casting/particulate leaching, replication, phase separation, 3D printing, fused deposition modeling, reactive foaming, electro spinning, and scCO₂ foaming constitutes these ranges. Supercritical carbon dioxide (scCO₂) foaming was first proposed by Mooney [3] to create porous PLGA and PLA scaffolds by the pressure quench method, which was proposed by Goel [9,10] to manufacture microcellular PMMA foams. There have been a number of followers, which worked on foaming of biodegradable polymers to create porous scaffolds by this method [7, 9~12]

This method consists in using CO₂ as a blowing agent for the polymer to create porosity. Carbon dioxide is used because it is relatively non-toxic, relatively inert, and non-combustible. Also, it has relatively reachable critical points ($T_c = 31^\circ\text{C}$, $P_c = 73.8$ bar) and is soluble in a lot of polymers. The solubility of CO₂ into polymers increases with pressure, which leads to work at supercritical pressures. Moreover, since the critical temperature of CO₂ is 31°C, it can be used to process thermally sensitive materials. On the other hand, the sorption of CO₂ into the polymers depresses their glass transition temperature which results in a polymer/gas solution. Typically, the foaming method consists in saturating polymer pellets with CO₂ at desired temperature and high pressure, followed by a rapid depressurization that causes the supersaturation. As a result of the supersaturation, the creation of nuclei occurs and the depressurization induced desorption from the polymer matrix and the phase change of the CO₂ provides the pore growth.

In the framework of this study, the creation of porous polylactic polymer by supercritical CO₂ foaming technique is investigated. According to the literature, it appears that the main parameters controlling the scCO₂ foaming are the concentration of CO₂ solubilized into the polymer and the depressurization rate of the pressure chamber. Therefore, the primary aim of this study is to compare the influence of the PLGA structure on the pore size of the foams created by scCO₂.

2. Experimental

2.1. Materials

Poly (lactic-co-glycolic acid), PLGA_{85:15} (D, L-lactide: glycolide) and PLGA_{50:50} copolymers have been purchased from Purac (PURASORB PDLG) and (Boehringer Ingelheim, Germany) respectively.

2.2. Preparation of Polymer Pellets and Foams

Firstly, approximately 0.150 g of polymer powder has been molded by compression at 150 bars and 60°C for 20 minutes in a compression mold for making pellets. Secondly, in order to create polymer scaffolds, 3 pellets of polymer have been simultaneously placed on metal grids inside the mixing chamber of the high pressure installation. The pressure chamber is then brought to the desired pressure and temperature. Then, polymer pellets have been saturated with CO₂ during a desired time. After that, the chamber has been depressurized with a constant depressurization rate to provide foaming of the polymer pellets.

2.3. Methods

The principles of the scCO₂ foaming by pressure quench method by supercritical carbon dioxide (scCO₂) were described by Goel and Beckman [9, 10].

In this work runs were carried out in a SEPAREX SF200 pilot (Separex Company, Nancy, France) Sub-cooled liquid CO₂ is pumped by a volumetric membrane pump (Milton Roy, maximum 5 kg/h), then heated until the desired temperature and continuously introduced into a cylindrical mixing chamber, which contains the polymer sample, with a flow-rate of 20 g/min. Pressure in the mixing chamber is adjusted by a backpressure regulator. Experiments are carried out in closed-loop configuration, i.e. after condensation, CO₂ is recycled at the pump inlet, in order to provide the stability. Temperatures and pressures are controlled in each unit of the pilot, pressure being limited to 300 bars and temperature around 60 °C. Temperatures of the pressure chamber and temperature of the heating fluid are recorded. Depressurization rates are controlled manually with the backpressure regulator [13] and a posteriori analyses of pressure recordings allow quantification of the depressurization rate (slope of the linear depressurization curve).

3. Physicochemical Analysis of Copolymers

3.1. Viscosimetry Study in Solution in CCl₄ at 25° C.

Determination of the molecular mass of the copolymers has been performed by viscosimetry. As shown on figure 1 the variations, with PLGA_{85:15} concentrations of the reduced specific viscosity and inherent viscosity are linear. The intercept point of both viscosity gives the intrinsic viscosity $[\eta] = 3.0$. By using the Mark-Houwink relationship $[\eta] = KM^a$ with $K = 0.000545$ and $a = 0.73$ [14, 15] we obtain the molecular mass of the copolymer: $M = 133,131$ g/mol.

By applying the same procedure to the PLGA_{50:50} we obtain its intrinsic viscosity $[\eta] = 0.60$ and molecular mass $M = 14,682$ g/mol.

Figure 1: Variation with PLGA_{85:15} ratio of reduced specific and inherent viscosities

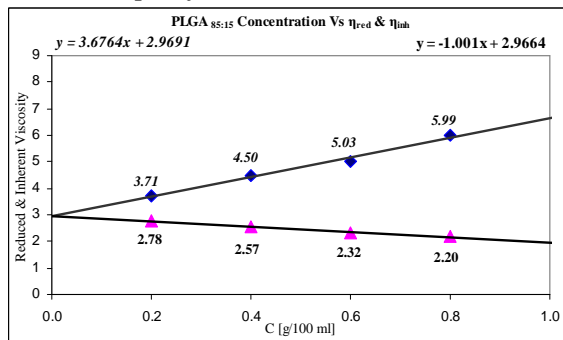
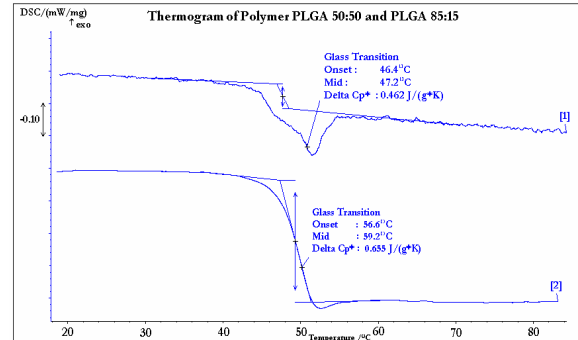


Figure 2: Thermograms of (1) PLGA_{50:50} and (2) PLGA_{85:15}



3.2. Thermal Analysis of Copolymers

The transitions of the two copolymers have been analyzed with a DSC 204 F1 Phoenix®. Three samples of each PLGA have been used to determine the glass transition temperature T_g and the step of heat capacity ΔC_p . As shown on figure 2, T_g are around 47°C and 57°C for PLGA_{50:50} and PLGA_{85:15} respectively and ΔC_{pT_g} are around 0.46 and 0.65 $J.g^{-1}.K^{-1}$ for PLGA_{50:50} and PLGA_{85:15} respectively.

4. scCO₂ Foaming

4.1. Factorial Design of Experiments (DOE)

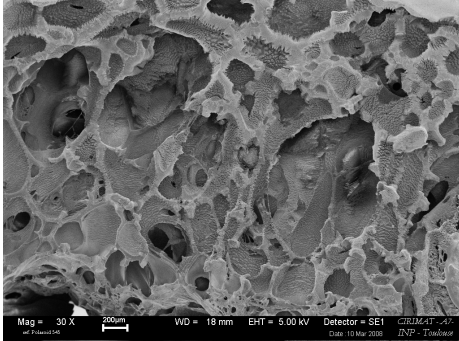
Previous works have shown that three parameters, the saturation temperature T_{sat} , the saturation pressure P_{sat} and the depressurization rate dP/dt , are the more influent parameters onto the pore distribution. So, all experiments were performed with a fixed a saturation time $t_{sat} = 60$ min and at different saturation temperature, pressure and dP/dt reported on Table 1 as given by a factorial DOE. As an example, pores observed by SEM for the PLGA_{85:15} are presented on figure 3. Analysis of images gives average pore diameters reported on table 1 for both PLGA.

Table 1: Experimental conditions and average pore diameters for both PLGA

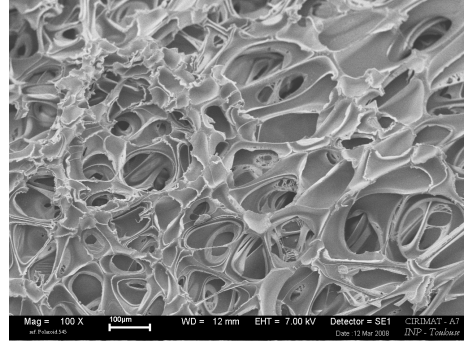
Exp.N ^f .	X ₁	X ₂	X ₃	T (°C)	P _{sat} (bar)	dP/dt (bar/s)	Average Pore Diameter (µm)	
							PLGA _{85:15}	PLGA _{50:50}
1	-1	-1	-1	35	100	1	264	153
2	1	-1	-1	60	100	1	187	142
3	-1	1	-1	35	300	1	89	30.3
4	1	1	-1	60	300	1	59	33
5	-1	-1	1	35	100	20	77	285
6	1	-1	1	60	100	20	89	114
7	-1	0.5	1	35	250	20	25	4
8	1	0.5	-1	60	250	1	36	27

The pores observed by SEM. Micrographs for PLGA_{85:15} for all the previous experiment conditions can be visualized on figure 3. Analysis of images gives average pore diameters reported on table 1.

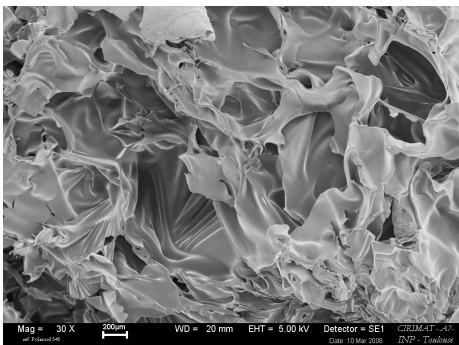
Figure 3: SEM Micrographs of PLGA_{85:15}



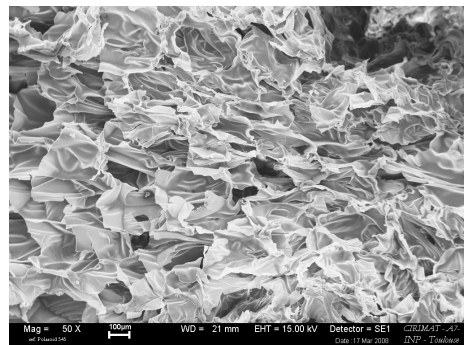
(1). $T_{\text{sat}}=35^{\circ}\text{C}$, $P_{\text{sat}} = 100\text{bar}$, $dP/dt = 1\text{bar}\cdot\text{s}^{-1}$



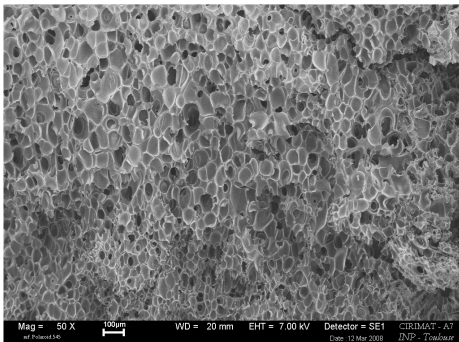
(5). $T_{\text{sat}}=35^{\circ}\text{C}$, $P_{\text{sat}} = 100\text{bar}$, $dP/dt = 1\text{bar}\cdot\text{s}^{-1}$



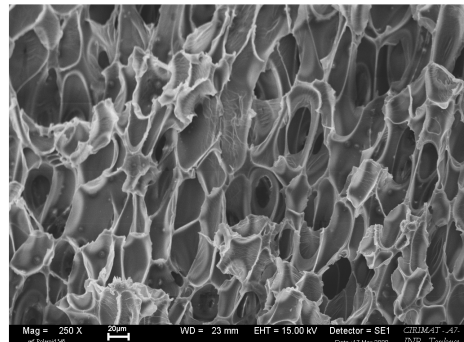
(2). $T_{\text{sat}}=60^{\circ}\text{C}$, $P_{\text{sat}} = 100\text{bar}$, $dP/dt = 1\text{bar}\cdot\text{s}^{-1}$



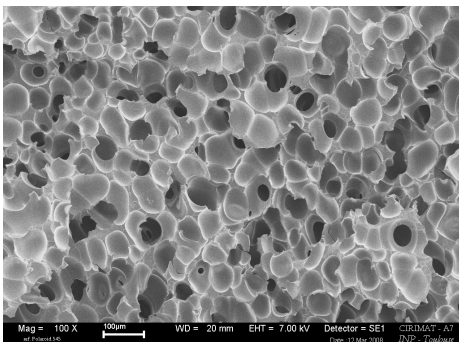
(6). $T_{\text{sat}}=60^{\circ}\text{C}$, $P_{\text{sat}} = 100\text{bar}$, $dP/dt = 20\text{bar}\cdot\text{s}^{-1}$



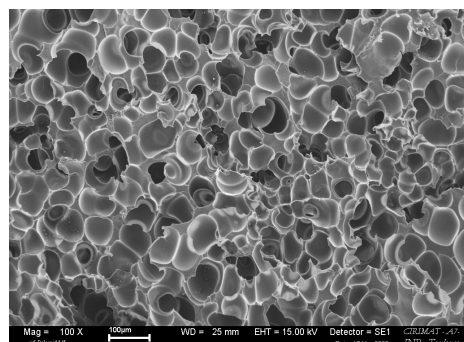
(3). $T_{\text{sat}}=35^{\circ}\text{C}$, $P_{\text{sat}} = 300\text{bar}$, $dP/dt = 1\text{bar}\cdot\text{s}^{-1}$



(7). $T_{\text{sat}}=35^{\circ}\text{C}$, $P_{\text{sat}} = 250\text{bar}$, $dP/dt = 20\text{bar}\cdot\text{s}^{-1}$



(4). $T_{\text{sat}}=60^{\circ}\text{C}$, $P_{\text{sat}} = 300\text{bar}$, $dP/dt = 1\text{bar}\cdot\text{s}^{-1}$



(8). $T_{\text{sat}}=60^{\circ}\text{C}$, $P_{\text{sat}} = 250\text{bar}$, $dP/dt = 1\text{bar}\cdot\text{s}^{-1}$

The same procedure applied on the PLGA_{50:50} copolymer has given results reported on table 1 and can be compared with the pore diameters of PLGA_{85:15}.

4.2. Discussion on the Effect of the Polymer Composition

Comparative results of the two factorial designs are presented on figure 4. Analyses of these results reveal that both pore diameter and porosity can be described by first order models: $y \sim = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3$ with coefficients reported on table 2.

Figure 4: Comparative Study of PLGA_{85:15} and PLGA_{50:50} Scaffolds

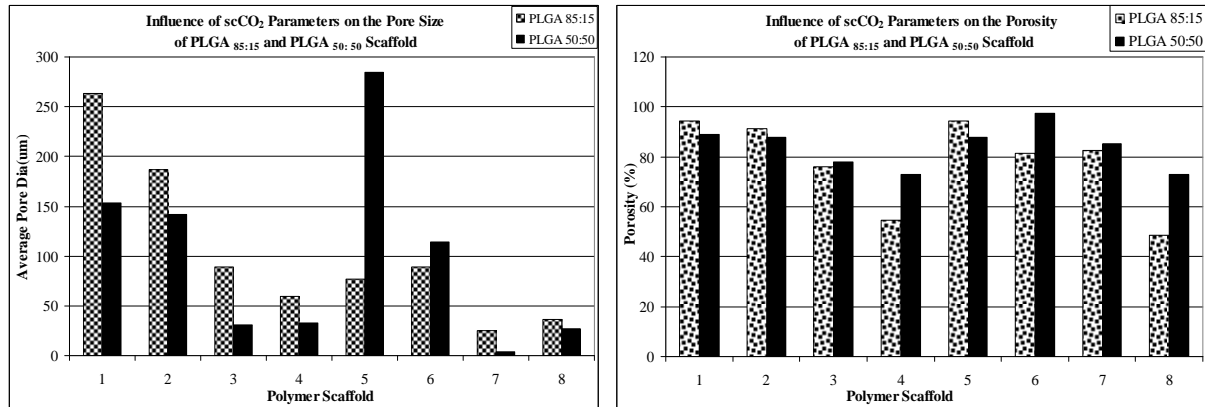


Table 2: Coefficients of various models

\hat{a}	Porosity		Pore diameter	
	PLGA _{85:15}	PLGA _{50:50}	PLGA _{85:15}	PLGA _{50:50}
a_0	75,5	85,4	98,6	48,7
a_1	-10,3	0,7	-10,8	-45,9
a_2	-14,3	-5,0	-53,6	-123,5
a_3	-1,3	4,0	-42,7	-35,7
a_{12}	-5,8	-1,2	7,5	0,9
a_{13}	-1,9	2,7	24,4	-38,7
a_{23}	0,7	1,8	26,4	-63,0

All experimental parameters, i.e. the pressure and the temperature of the saturation and the rate of depressurization have an influence on the final pore size of the scaffolds. For porosity and pore diameter, the most important parameter is the factor 2 *i.e.* the pressure of saturation. Other factors have a contrasted behavior. The factor 1 *i.e.* the temperature of saturation is only influent on the porosity of PLGA_{85:15} and on the pore diameter of PLGA_{50:50}. As for the factor 3 *i.e.* the depressurization rate, it is mainly influent on the pore diameter of PLGA_{85:15} and on the porosity of PLGA_{50:50}. Moreover, for PLGA_{85:15} all factors coefficients are negative: the porosity and the pore diameters are growing by decreasing of process parameters. For PLGA_{85:15} the coefficient a_3 is positive for porosity and negative for pore diameter: it isn't possible to increase simultaneously the porosity and pore diameter. Differences between both copolymers are linked to differences of solubility of CO₂. Our experimental and bibliographic research study showed that the solubility into the polymer increases while the LA content increases in a PLGA co-polymer. This behavior has been explained by the existence of an extra methyl group in LA as compared to GA [16], which, according to the authors, can drive to two opposite phenomena: firstly, it decreases the effect of the CO₂ interaction with the carbonyl group of the polymer, and secondly, it creates more available free volume for CO₂ molecules. Besides, Kazarian [17] have found that the

interaction of CO₂ with polymers can also be explained by chemical interactions, and CO₂ can behave like a Lewis acid. As a consequence, LA/GA ratio of a PLGA co-polymer is an important parameter to control the pore diameter in a foaming process.

For low depressurization rates, when the lactic acid content increases in PLGA, the pore size increases as well. This behavior can be attributed to the greater capacity of CO₂ to penetrate into the polymer with the increasing amount of LA. Actually, one can expect that since the CO₂ concentration is greater in a high lactic acid containing PLGA, the nucleation rate must be greater (which means lower pore size). However, even if the number of pores is determined by the saturation period and the concentration of CO₂ inside the polymer, the final pore size of the scaffold is mostly related to the period of desorption. During desorption, a number of phenomena occurs; the desorption-diffusion, the swelling of the polymer due to the growing of the pores, the coalescence of growing pores, the vitrification and the increasing of the glass transition temperature of the polymer which is related to the desorption of CO₂. Furthermore, during the saturation period, the more CO₂ is sorbed into the polymer, the more depression of T_g occurs. The more plasticized polymer, which contains more CO₂, will take more time to desorb, and will vitrify later than a polymer which sorbed less CO₂.

Consequently, the polymer which tends to sorb less CO₂ will vitrify sooner, and this will stop the growth of the pores. On the other hand, we must underline that different co-polymer like PLGA_{50:50}, PLGA_{85:15} have different glass transition temperatures and also different ΔC_p at the glass transition, which affects the depression of T_g during the saturation, and the increase of T_g during the desorption. These differences in T_g and $\Delta C_{p(T_g)}$ must be considered in order to achieve a complete analysis of the phenomena.

References:

- [1] BECKMAN, E. J., *J. Supercritic. Fluids*, Vol. 28, **2004**, P-121~191.
- [2] FWU-LONG MI, SHIN-SHING SHYU, YI-MEI LIN, YU-BEY WU, CHIH-KANGPENG, YI-HUNG TSAI, *Biomater.* Vol.24, **2003**, P-5023~5036
- [3] MONEY, D. J., BALDWIN, D. F., SUHT, N. P., VACANTIS, J. P., and LARGER R., *Biomater.*, Vol.17, **1996**, P1417~1422.
- [4] SINGH, L., KUMAR, V., and RATNER, B.D., *Biomater.*, Vol.25, **2004**,P-2611~2617
- [5] COTUGNO, S., DI MAIO, E., MENSITIERI, G., IANNACE, S., ROBERTS, G.W., and CARBONELL, R.G., *Ind. Eng Chem. Res*, Vol.44, **2005**,P-1795~803
- [6] JENKINS, M.J., HARRISON, K.L., SILVA, M., WHITAKER, M.J., SHAKESHEFF, K.M., and HOWDLE, S.M., *Euro Polym Journal*, Vol.42, **2006**,P-3145~51.
- [7] TSIVINTZELIS, I., PAVLIDOU, E., and PANAYIOTOU, C., *J Supercritic. Fluids*, Vol.42, **2007**, P-265~72.
- [8] WANG X., LI, W., and KUMAR, V., *J Biomater.*, Vol.27, **2006**, P-1924~9.
- [9] GOEL, S. K. and BECKMAN, E. J., *Polym. Eng. and Sci.*, Vol. 34, **1994**, P-1137~1147.
- [10] GOEL, S. K. and BECKMAN, E. J., *Polym. Eng. and Sci.*, Vol.34, **1994**, P-1148-1156.
- [11] SHAKESHEFF, K. M., QUIRK, R.A., FRANCE, R. M. and HOWDLE, S. M., *Current Opinion in Solid State and Materials Science*, Vol.8, **2004**, P-313~321.
- [12] REVERCHON, E. and CARDEA, S., *J. Supercritic. Fluids*, Vol. 40, **2007**, P-144~152.
- [13] CAMY S., and CONDORET, J.S., *J. Supercritic. Fluids*, Vol. 38, **2006**, P-51~61.
- [14] SCHINDLER, A., and HARPER, D., *J. Polym. Chem. Edn.*, Vol.17, **1979**, P-2593~9.
- [15] VÄLIMAA, T.; and LAAKSOVIRTA, S., *Biomater.*, Vol.25(7-8), **2004**,P-1225~32.
- [16] LIU, D. and TOMASKO, D. L., *J. Supercritical Fluids*, Vol.39, **2007**, P-416~425
- [17] KAZARIAN, S. G., VINCENT, M. F., BRIGHT F. V., LIOTTA C. L., and ECKERT C. A., *J. Am. Chem. Soc.*, Vol-118, **1996**, P-1729~1736.