Preparation of Porous Polycaprolactone Tubular Matrix by Salt Leaching Process

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ABSTRACT: Salt leaching technique has been employed to produce polycaprolactone (PCL) based porous tubular matrix. Salt was dispersed into PCL solution in chloroform and the subsequent leaching of salt was accomplished in water. The effect of salt and polymer concentration on the mechanical properties, porosity and surface characteristics was studied. It was observed that salt concentration has significant influence over crystalline nature of the scaffold. The porous structures became more interconnected as the salt concentration increased. This process yields a tubular porous matrix with maximum porosity of 61%, strength of 1.84 (±0.04) MPa and tensile strain of 120 (±7.94). © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: polycaprolactone; salt leaching; porous matrix; tubular

INTRODUCTION

Polymeric porous matrixes found promising field of application in filtration,[1–3] tissue engineering,[4] and healthcare.[5,6] Polymeric porous structures can be prepared by various established methods such as phase separation method. The principle of phase separation is based on the separation of solid polymeric phase from its liquid solution phase. Phase separation is achieved by lowering the solubility of polymer. Insolubility of polymer can be induced by the incorporation of nonsolvent in the polymer solution. The basic principle of nonsolvent induced phase separation (NIPS) is used in various methods for porous matrix formation. In conventional NIPS or coagulation method, polymer solution is dipped into nonsolvent containing coagulation bath for phase separation. Spray phase separation (SPS) method is a NIPS aided method, where polymer solution and coagulant are sprayed simultaneously to form a porous scaffold.[7–9] Kennedy et al.[7] showed the formation of porous film of polyurethane with 70–88% porosity depending on the ethanol concentration in the coagulant mixture. In a different approach, porous architecture is obtained by temperature-induced phase separation (TIPS). Solubility of polymer in the solution is reduced by lowering the temperature, which leads to phase separation and eventually porous architecture.[10,11] Freeze drying produces porous film by using the principle of TIPS. In this method, by lowering the temperature, solubility of the polymer decreases and produces polymer rich and lean phases. Temperature of the system is lowered down to the freezing point of the solvent, leading to solvent solidification. Those solidified solvents are then removed from the system by applying vacuum at the same lower temperature, leaving a porous structure.[12]

The simplest process of porous film formation is casting polymeric substances along with porogen into a custom designed cast. To achieve a porous structure, polymer melt or solution along with porogen is casted into a predesigned mold. Subsequently, after solidifying the liquid, the porogen is leached out by dissolving it into a solvent. The main advantage of porous matrix preparation by salt leaching lies in simplicity of the method, along with ease to get an interconnected porous structure. Various combinations of polymers and porogens used are poly(DL-lactic-*co*-glycolic acid) (PLGA), poly(trimethylene carbonate), poly(lactic-*co*-caprolactone), deacrylated polycaprolactone as polymer and sugar, sodium chloride, wax as porogens.[13–18] Hou et al.[16] have combined solvent casting and porogen leaching method with polymer coagulation for getting a homogeneous precipitate of porogen and polymer mixture. This isolated precipitate then compressed mould on a hot press to achieve desired shape and followed by porogen leaching. Reignier et al.[19] combined salt particulate leaching with polymer leaching from a cocontinuous blend of polycaprolactone

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(PCL) and polyethylene oxide (PEO) for the preparation of interconnected PCL porous scaffolds with a bimodal pore size distribution.

PCL is a widely used biodegradable semi crystalline polymer of polyester family. It has low glass transition temperature (T_g) of –60°C and low melting temperature (T_m) of 59–64°C.[20] Low T_g gives it an exceptional flexibility in comparison to other biodegradable synthetic polymers, such as polylactic acid (PLA), polyglycolic acid (PGA). PCL is low cost polymer and it is easily soluble in common solvents, such as chloroform, dichloromethane, hexafluoro-2-propanol, tetrahydrofuran and toluene. PCL is U.S. Food and Drug Administration (FDA) approved for drug delivery usage and also widely used for other biomedical applications, such as implants, suture, tissue engineering.[20]

One of the most promising fields of application of porous tubular matrix is tissue engineering. Tissue engineering has been defined as an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes, which restore, maintain, or improve tissue function.[21] Polymeric porous films are preferred as cell supporting porous matrix in tissue engineering. Tubular structures are adapted in soft tissue regeneration, such as, nerve regeneration,[22] blood vessel regeneration.[13] Precise designing of the scaffold is extremely crucial for guiding cells to grow and proliferate into a three dimensional tissue structure with proper diffusion of oxygen, nutrients, and metabolic wastes.[20] The structure property correlation in scaffold preparation needs to be investigated in a proper manner so that the influence of the structure on material characteristics may be precisely monitored. In our studies, we have prepared porous PCL scaffold by salt leaching approach aiming potential use as a tubular scaffold for blood vessel regeneration. The influence of PCL concentration and porogen content on the structure of the scaffold has been ascertained.

EXPERIMENTAL

Materials

Polycaprolactone ($M_w \sim 80{,}000$), was procured from Aldrich. Chloroform (Qualigens®) was used as solvent and was double distilled prior to use. Sodium chloride supplied by Fisher Scientific was used as porogen.

Salt preparation

Sodium chloride salt was ball milled in ball milling machine (Retsch PM 100) at 300 rpm with 20 iron balls of 6.91 g weight, for 2 h. Particle size was measured by the particle size analyzer (Brook Haven 90 plus particle size analyzer) by dispersing them into acetone. The average particle size was 1.8 μm.

Porous tubular matrix preparation

In this work a combination of dip coating and salt leaching method is used for preparing porous tubular matrix. PCL solutions of three different concentrations of 2.5, 5, and 7.5% (w/w) were prepared in chloroform. The salt concentration was varied in the range of 33–75%. Salt was added to PCL solution under constant stirring. A 5 mm diameter glass rod was placed manually in the polymer solution for 5 s and was removed and dried in air. These samples with the glass rod were kept overnight in distilled water, under mild stirring at 200 rpm to remove salt. Samples were removed from the glass rods, followed by the vacuum drying.

Material characterization

Viscosity measurement

Viscosity of PCL solution was measured using a Brookfield Viscometer. Measurements were taken at two different rpm, at 10 and 20 rpm. Molecular weight of the polymer was calculated using an Ubbelohde Viscometer.

Scanning electron microscopy (SEM)

A ZEISS EVO 50 scanning electron microscope (Carl Zeiss, North America), at a tension of 30 kV, was used to monitor surface morphology. Samples were coated with gold by Polaron gold sputter coater system prior to the scanning.

Differential scanning calorimetry (DSC)

DSC analysis was carried out using a PerkinElmer DSC 7 system in the temperature range of 20–100°C at a rate of 10°C/min under nitrogen atmosphere.

X-ray Diffraction (XRD)

XRD were recorded with a Wide angle X-ray Diffractometer (PANalytical) with accelerator counter at a scanning rate of 0.066°/min and within the scanning region of 2θ, 5–40° with Cu Kα radiation ($\lambda = 1.5418$ Å) and irradiation conditions of 40 kV and 40 mA.

Tensile strength

The tensile strength of the samples was measured with a computer controlled Instron 3401 equipped with mechanical grip. The tensile measurements were performed with 1 kg (9.81 N) load cell at a crosshead speed of 50 mm/min and a gauge length of 20 mm and width of 5 mm. Five specimens of each sample were tested. The average thickness of the outer diameter of each tube was measured by

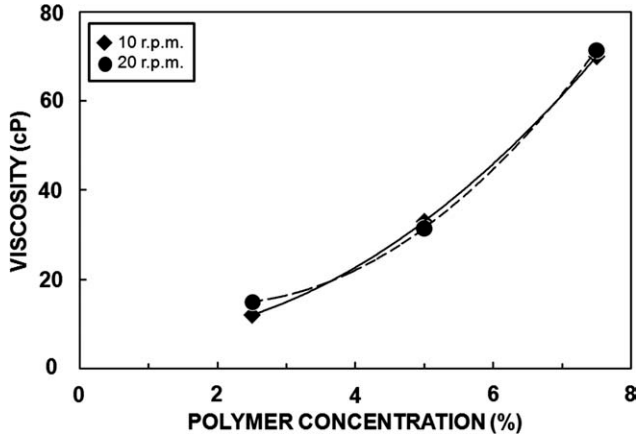

Figure 1 Variation of viscosity with polymer concentration.

SEM and free photo editor software GNU Image Manipulation Program (GIMP). None of the samples broke close to the grips.

Porosity measurement

The porosity of the samples was calculated as per the following relation:[23]

$$\text{Porosity } (\%) = (V_m - V_0)/V_0 \times 100$$

where, V_m and V_0 are the specific volumes of the salt leached sample and nonporous films, respectively.

RESULTS AND DISCUSSION

Effect of polymer concentration on mechanical and morphological properties of tubular porous matrix:

The viscosity of PCL solution as a function of concentration is presented in Figure 1. Increase in the polymer concentration provides higher number of polymer chains, causing amplified number of chain entanglement points which leads to increased viscosity. With the increasing polymer concentration, viscosity of the solution also increased from 12 cP for 2.5% to 70 cP for 7.5% PCL chloroform solution.

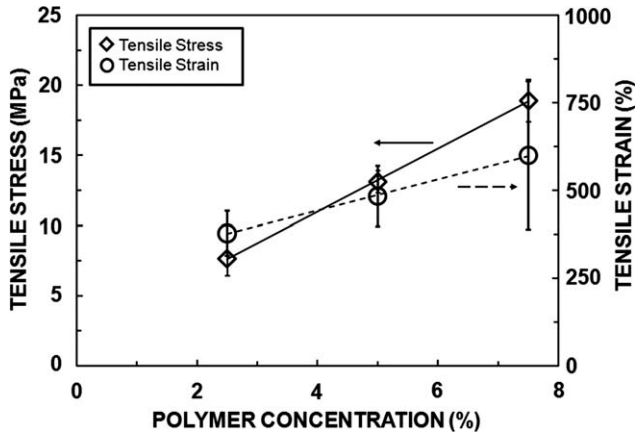

Figure 2 Variation of mechanical properties with polymer concentration.

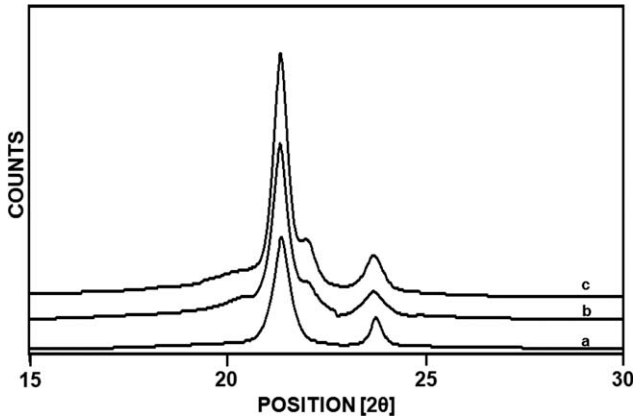

Figure 3 X-ray diffraction patterns of scaffolds prepared with different PCL concentrations (w/w) (a) 2.5%, (b) 5%, and (c) 7.5%.

The polymer concentration has significant influence over the mechanical properties as presented in Figure 2. Tensile strength shows increasing trend with the increasing PCL concentration. The increase in polymer concentration leads to an increase in number of load bearing polymeric chains, which results in an increasing tensile strength. The effect of polymer concentration on tensile strain also shows increasing trend (Fig. 2). Higher polymer concentration gives higher number of chain entanglement points, which leads to higher chain restriction, causing increased tensile strain. Native human arterial tissue has a tensile strength in the range of 0.78–1.37 MPa, and tensile strain between 65–83%.[24] All three scaffolds show better mechanical properties than the native tissue. Samples prepared out of 5% PCL chloroform solution show better homogeneity of the scaffold, evidenced by the low standard deviation of the mechanical data.

Effect of polymer concentration on crystal structure of tubular porous matrix:

The XRD of PCL scaffolds prepared under different polymer concentration are presented in the Figure 3. The XRD peak intensity of the scaffolds increases for the one prepared at higher polymer concentration. This is the indication of better molecular chain orientation and crystallization of polymer chains at higher polymer concentration, which leads to relatively higher crystallinity at higher polymer concentration from 44 to 47%. According to these mechanical, morphological and crystallographic studies, 5% PCL concentration was used for further studies.

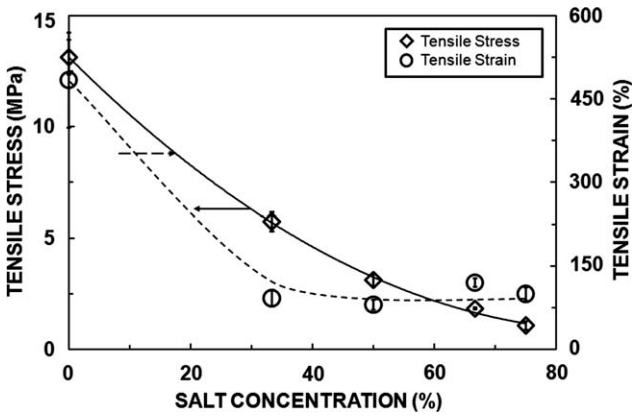

Figure 4 Variation of the mechanical properties with the salt concentration (polymer concentration 5%).

Effect of salt concentration on mechanical and morphological properties of tubular porous matrix

The addition of salt and its concentration has significant influence over the tensile and morphological features of the scaffold. The effect of salt incorporation on the mechanical properties of the scaffolds, prepared from 5% PCL solution is presented in Figure 4. Salt leaching introduces the porosity in the structure, which abruptly reduces the tensile strength. Tensile strength shows continuous decrease with the increase in salt concentration. Moreover, the larger porosity at higher salt concentration leads to the disruption of the continuity in the polymer structure, which makes the structure brittle and causes sudden fall in tensile strain values, which remains almost identical for further increase in salt concentration. This can be explained by the competing nature of two separate phenomena of pores. Pores act as the weak points in the structure, causing sudden fall in tensile strain, which is compensated by the pore stretching at the higher porosity, leading to almost same strain.

The effect of salt concentration on surface morphology and porosity of the sample is shown in Figures 5 and 6. With the increase in salt concentration, pores become more pronounced and interconnected to each other. The overall porosity of the scaffolds also increases with the salt concentration. These scaffolds show 61% porosity prepared from 5% PCL chloroform and 67% salt dispersion.

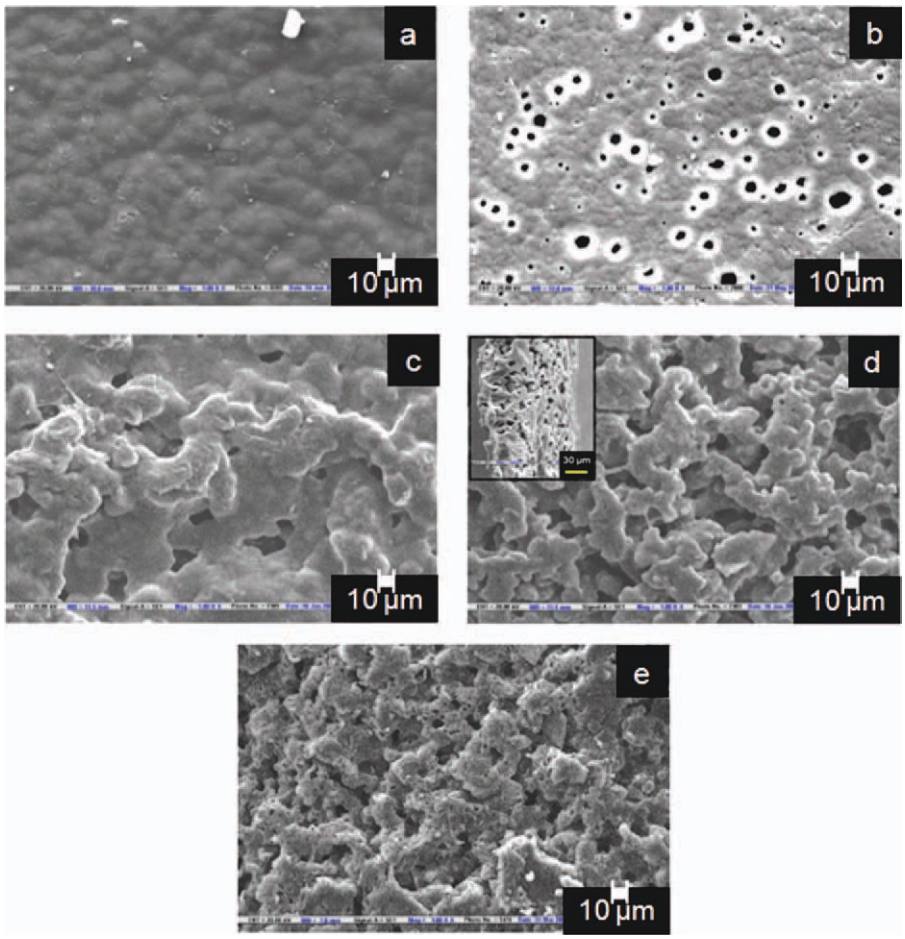

Figure 5 SEM of scaffolds (a) 0% salt, (b) 33% salt, (c) 50% salt, (d) 66% salt and (e) 75% salt (polymer concentration 5%). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

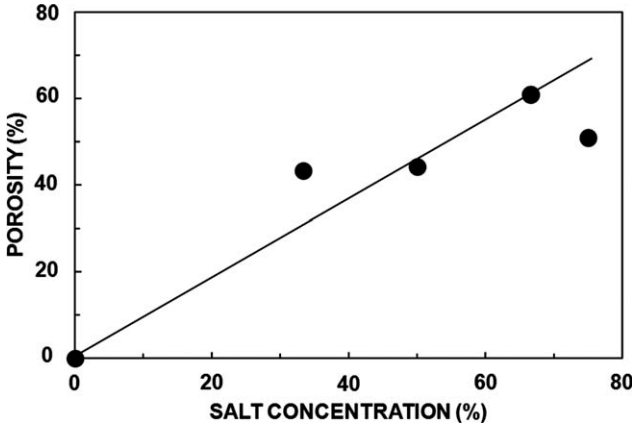

Figure 6 Variation of porosity with salt concentration (polymer concentration 5%).

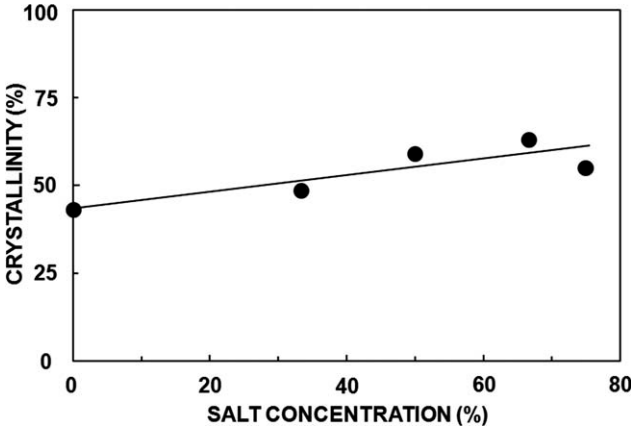

Figure 8 Variation of crystallinity with salt concentration (polymer concentration 5%).

Effect of salt concentration on crystal structure of tubular porous matrix

The effect of salt addition on the X ray diffraction pattern of the scaffolds is shown in the Figure 7. The crystallinity calculated from these plots is presented in Figure 8. With the increase in salt concentration, the crystallinity was slightly decreased from 77 to 63%. This phenomenon is supported by the work carried out by Baji et al.[25] where they incorporated hydroxyapatite with PCL and monitored its effect on the crystallinity. They reported a decrease in the peak intensity of PCL with the increase of filler along with unmodified basic crystal structure of PCL. In our study, PCL shows peaks at around 21° and 23.8° 2θ positions in XRD. The addition of 33% salt showed unmodified crystal structure with a lower crystallinity. However, further increase in salt concentration led to the origin of additional peaks in XRD (Fig. 7). With the increase in salt concentration beyond 33%, two extra peaks at 14° and 17° in 2θ position emerge along with the usual peaks of PCL.

It seems that crystal structure modification is the significant feature during the sample preparation. It is observed that the crystal structure of polyketone $(\text{—CH}_2\text{—CH}_2\text{—CO—})_n$ is isomorphous to polyethylene (PE) ($a = 7.97$ Å, $b = 4.76$ Å, $c = 7.57$ Å), where C=O groups of two chains are located at the same height in the unit cell.[26,27] Because of the higher concentration of methylene groups, flexibility of the ester groups and different heights of carbonyl groups of two chains in the unit cell causes a defect in the single primary crystal structure of polyethylene. This results in a higher "*c*" value for the polyesters of $(\text{—CH}_2\text{—CH}_2\text{—CO—O—})_n$ type than PE crystal structure. This causes tilts in the polymer chains along its fiber axis. Therefore, for PCL a rotation of chain of 28° with respect to the "a" axis happened instead of 49° rotation as in PE, leading to a PCL unit cell dimension of $a = 7.47 \pm 0.03$ Å, $b = 4.98 \pm 0.03$ Å, $c = 17.05 \pm 0.04$ Å.[26,27]

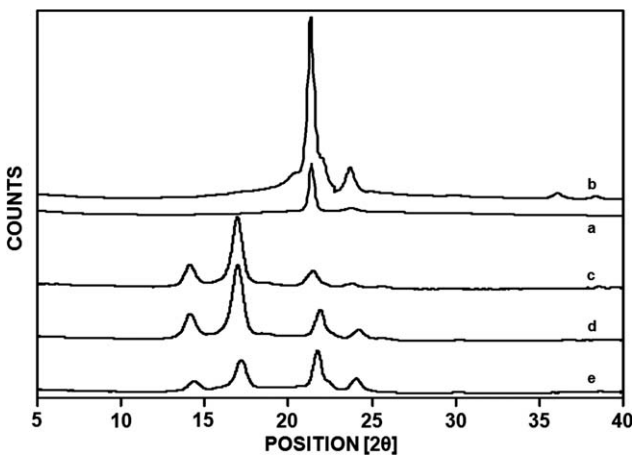

Figure 7 X-ray diffraction patterns of scaffolds (a) 0% salt, (b) 33% salt, (c) 50% salt, (d) 66% salt and (e) 75% salt (polymer concentration 5%).

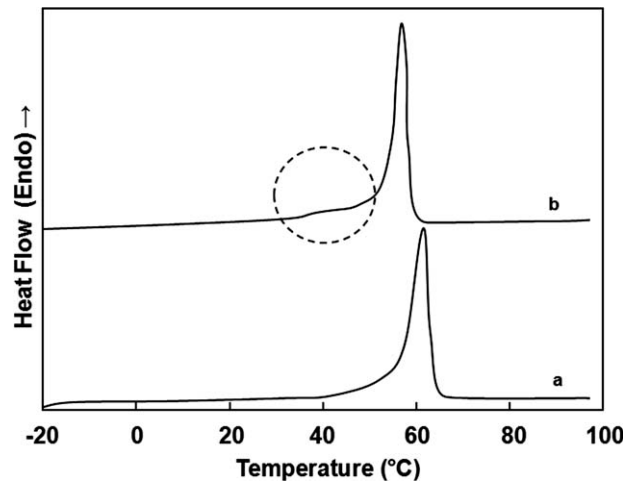

Figure 9 DSC thermogram of (a) Virgin PCL and (b) scaffold prepared from 75% salt (polymer concentration 5%).

It is being reported that polyethylene itself has two different crystal forms, one stable orthorhombic form and the other is metastable monoclinic form. Depending on the z value, $(-(CH_2)_z-CO-O-)_n$ type polyesters also show these two types of crystal forms. For odd values of z, orthorhombic crystal structure and for even values of z, monoclinic form is obtained. But, there are a number of exceptions too. For polypropiolactone ($z = 2$), two different crystal forms, extended and helical are found. For polypivalolactone, by different cooling rate three different crystal forms, i.e., α, β, γ are formed. All these examples gives support to the second crystal form of PCL, which gives four different peaks in the XRD pattern.[26,27] By comparing the XRD patterns of these two different crystal structures of PCL with known XRD patterns of PE, it is evident that the new structure may be a monoclinic structure. The XRD observations are also supported by the DSC studies. Compared to the DSC thermogram of normal PCL [Fig. 9(a)], the DSC thermogram of salt leached PCL scaffold [Fig. 9(b)] shows a slightly modified melting temperature at 57 from 62°C with a second submerged melting peak. This is evidence of a new conformation of the PCL polymeric chain due to the salt leaching process as inferred from the XRD patterns.

CONCLUSIONS

The porous PCL scaffolds may be prepared by salt leaching approach, where the porosity and the tensile strengths of the scaffolds are significantly influenced by the salt concentration. A maximum porosity of 61% is achieved for the salt concentration of 67%. During the salt leaching process, basic PCL crystal structure has been modified from stable orthorhombic form to metastable monoclinic form. At low salt concentration, the transformation is not visible. However, as the salt concentration increases, the monoclinic form is evident. The surface morphology and porosity is also affected by the salt content. The pores become more pronounced and larger in size at high salt concentration. Consecutively, the porosity also increases at higher salt concentration. It may be visualized that, a mechanically compatible porous tubular framework with tensile strength of 1.84 (±0.04) MPa and tensile strain of 120 (±7.94)%, can be obtained from 5% PCL chloroform solution with 67% salt dispersion.

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