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Fabrication and characterization of poly(3-hydroxybutyrate) gels using non-solvent-induced phase separation

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ABSTRACT

Chloroform (CF) solutions of biodegradable poly(3-hydroxybutyrate) (PHB) formed organogels upon the addition of tetrahydrofuran (THF) as a non-solvent at room temperature. Conformational changes of PHB chains during gelation were monitored *in situ* by time-dependent infrared spectral (IR) measurements. Analysis of the IR spectra for different wavenumber regions (C—H, C═O, and C—O—C stretching vibration regions) showed that PHB chains underwent a molecular transition from a random coil to a helix indicating formation of a crystalline phase following a liquid–liquid phase separation during gelation. Applying solvent exchange and freeze-drying processes to the as-produced organogels allowed fabrication of nanofibrous PHB monoliths with high porosity (~97%) and surface areas up to 72.1 ± 0.6 m^2/g. It was shown that by varying the solvent mixture composition (THF/CF ratio), the gelation kinetics, crystallization behavior, nanofibrous structure, pore character, surface area, and the mechanical properties of PHB aerogels, were effectively controlled.

1. Introduction

Polymer gels are three-dimensional (3-D) polymer networks swollen by large quantities of solvent. Gels are phenomenologically defined as complex systems containing large quantities of fluid but exhibiting solid-like properties. Cross-linking of a polymer network can also be accomplished via either a permanent chemical bonding or weak supramolecular interactions between polymer chains [1]. Poly(3-hydroxybutyrate) (PHB) is a semi-crystalline linear polyester synthesized by micro-organisms, and it has become one of the most interesting biomaterials owing to its excellent biodegradability and biocompatibility [2–4]. Organogels made of PHB and its copolymers have been previously prepared by phase-separation methods [5–13]. These organogels become highly porous monoliths by careful removal of the solvents, which makes them potential materials for high-quality foams and scaffolds [8–13]. The thermally induced phase-separation method (TIPS) represents one of the most important approaches used for fabricating polymer gels [8–24]. By making appropriate changes to the temperature of a homogeneous polymer–solvent solution, polymer–solvent affinity decreases. This induces liquid–liquid phase separation of the interpenetrating network structure of a polymer-rich phase and a polymer-lean phase. In the case of semi-crystalline polymers, crystallization occurs in a polymer-rich phase and whole solution gels because micro-crystallites can serve as junctions for a 3-D network [25–31,35]. The thermally induced liquid–liquid phase separation followed by gelation of PHB in dimethylformamide (DMF) or 1,4-dioxane was reported to have taken place by applying a thermal stimulus comprising a heating (to 100 °C) and cooling (to/below room temperature) cycle [5–11]. Liquid–liquid phase separation and subsequent gelation from polymer solutions can be induced by not only TIPS but also by adding a non-solvent, which is called the non-solvent-induced phase separation (NIPS) method [28–35]. A polymer-rich phase consists of a polymer and a portion of solvent. A polymer-lean phase contains a non-solvent and the remaining solvent. The polymer-rich phase develops the skeleton of the monolith, whereas the polymer-lean phase flows through the matrix, resulting in porous channels within the monolith. The addition of 1,4-dioxane as the non-solvent to PHB/chloroform (CF) caused the thermodynamic de-mixing of the system into two different phases, the PHB-rich phase and PHB-lean phase [12,13]. In this case, the liquid–liquid phase separation and subsequent gelation occurred at 4 °C producing stable formation of nanofibrous gels. This process can be considered to be a combination of NIPS and TIPS techniques because phase separation occurs at

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temperatures other than room temperature. In this study, we prepared PHB organogels by adding tetrahydrofuran (THF) as the non-solvent to PHB/CF solutions. The phase separation and gelation took place under ambient conditions (25 °C). This process can be considered to be a form of NIPS where no thermal stimulus or below-ambient temperatures were required. The application of NIPS to PHB/CF/THF systems has not been studied in detail, whereas there have been extensive studies on TIPS of PHB/1,4-dioxane and PHB/CF/1,4-dioxane [8–13]. Conformational changes of PHB with time were monitored *in situ* during the gelation of the PHB/CF/THF solutions and analyzed for the first time by Fourier transform infrared (FTIR) spectrometry. Recently, nanofibrous scaffolds have been increasingly exploited for medical applications owing to their high surface-to-volume ratios and their ability to mimic the natural morphology of extracellular matrices (ECMs) [8–24,36]. In the current study, applying solvent exchange and freeze-drying processes to the as-produced organogels facilitated the fabrication of nanofibrous PHB aerogels with a high porosity (~97%). To our knowledge, this is the first report on the production of nanofibrous structures from PHB/CF/THF solutions. Solvent mixture compositions (THF/CF ratio) were one of the important processing parameters for controlling the nanofibrous structure, the surface area, crystalline form, as well as the mechanical properties, of the PHB monolithic aerogels and were studied in detail.

2. Experimental section

2.1. Materials

Poly(3-hydroxybutyrate) with a mass-average molecular mass of $M_w = 4.37 \times 10^5$ was purchased from Sigma Aldrich. Analytical-grade chloroform (CF) and tetrahydrofuran (THF) were purchased from Sigma-Aldrich and used as received.

2.2. Preparation of PHB organogels

CF is a well-known and good solvent for PHB. THF does not dissolve PHB but it is miscible in CF. THF was selected as the non-solvent for the PHB/CF solutions in order to achieve NIPS. The PHB solutions were prepared by dissolving PHB polymer in CF under stirring at 95 °C then cooling the solutions down to room temperature. The non-solvent, THF, was subsequently added to the PHB solutions at room temperature. The mixed solutions were kept at 25 °C using heating blocks during the phase separation followed by gelation. In this study, the polymer concentration (ϕ) was defined on the basis of the total volume of chloroform and THF. The PHB concentrations were adjusted from $\phi = 0.005$ g/mL to 0.03 g/mL. The properties and structure of the organogels and aerogels were significantly affected by the solvent mixture composition. The ratio of non-solvent/solvent (THF/CF v/v) was varied from 0/100 to 100/0. Gelation was confirmed by the formation of self-supporting samples that did not flow when inverted 180°. The gel transition time determined by the inversion tests depended on the solvent composition and PHB concentration.

Table 1
Gel formation and transition time of PHB in THF/CF.

ϕ (g/mL)	THF/CF (v/v)	Gel formation	Transition time
0.03	0/100	X	N/A
	10/90	X	N/A
	20/80	X	N/A
	30/70	O	30 h
	40/60	O	35 min
	43/57	O	18 min
	50/50	O	8 min
	60/40	O	1 min 45 s
	70/30	O[a]	1 min 30 s
	80/20	O[a]	1 min 10 s
	90/10	X	N/A
	100/0	X	N/A
0.02	43/57	O	25 min
0.01	43/57	O	70 min
0.005	43/57	O	160 min

Samples were placed in a heating block at 25 °C during gelation. X indicates that gelation was not observed, O that gelation was observed and O[a] that gel formation was non-uniform.

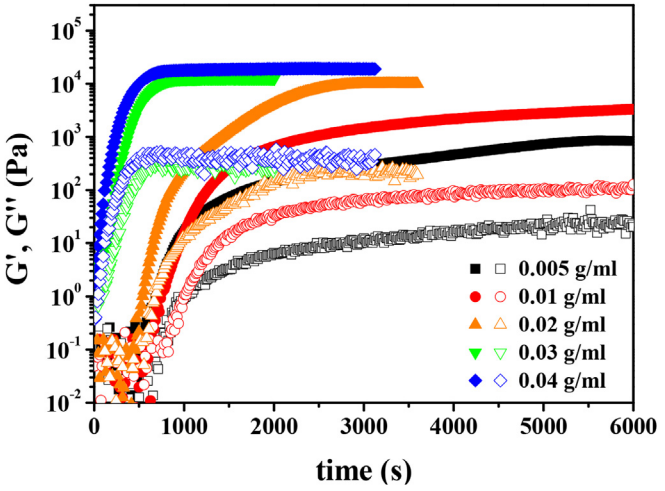

Fig. 2. Time sweep test of PHB solutions as a function of PHB concentration at a fixed solvent composition of THF/CF = 40/60. The frequency was maintained at 1 rad s^{-1}. Closed symbols denote G′ and open symbols denote G″.

2.3. Preparation of PHB aerogels

The wet, aged gels were removed by breaking their glass vessels and were immediately immersed in methanol. The solvent (CF) and non-solvent (THF) of the monolithic organogels were sequentially extracted by soaking the gel first in methanol for 2 days and then in

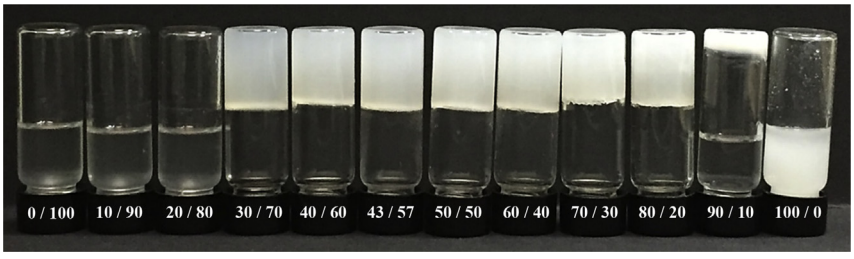

Fig. 1. The photographs of PHB solutions with different THF/CF (v/v) ratios. The polymer concentrations (ϕ) were fixed to 0.03 g/mL.