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Physically Cross-Linked Cryogels of Linear Polyethyleneimine: Influence of Cooling Temperature and Solvent Composition

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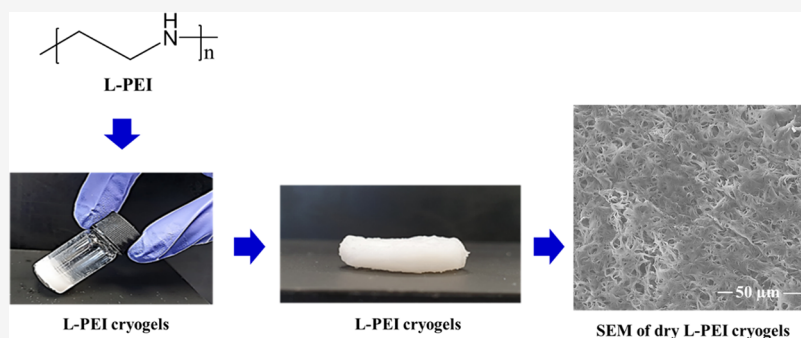
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ABSTRACT: Physically cross-linked cryogels can be prepared using linear polyethyleneimine (L-PEI) and a freeze-thawing technique. L-PEI was synthesized by hydrolysis of poly(2-ethyl-2-oxazoline) under acidic conditions. Dissolution of L-PEI in deionized water was achieved at 80 °C and resulted in a transparent solution, leading to the formation of an opaque gel upon freezing and subsequent thawing. The cryogels exhibited reversibility, and after heating at 80 °C, they formed a clear solution due to the melting of the crystalline domains of L-PEI. The effects of different cooling temperatures and the use of various solvent compositions on L-PEI gelation were also studied. L-PEI cryogels were prepared by freezing aqueous solutions to various temperatures (−196, −80, −30, and 0 °C) for 3 h and subsequent thawing at 25 °C for 24 h. Gel rigidity correlated with the freezing temperature and was strongest when cooled down to −196 °C, consistent with determinations of the degree of crystallinity in the gels, the enthalpy of fusion, and rheological behavior. The effect of solvent mixtures on the crystallinity and rheological properties of L-PEI cryogels was also investigated. Water/ethanol mixtures containing a higher proportion of ethanol significantly reduced the strength, viscosity, and degree of crystallinity of the L-PEI cryogels. Thus, by controlling the freezing temperature or modifying the solvent, L-PEI cryogels can be designed with desired mechanical properties for applications ranging from cell immobilization and tissue culture scaffolds to drug delivery systems or antimicrobial wound dressings.

1. INTRODUCTION

Polymeric gels are extensively used for biomedical applications due to their flexibility, high water content, biocompatibility, and softness.[1] They are also widely used in drug delivery systems, chromatography, biomedical scaffolding, and wound-healing products and as immobilization matrices. Gels are defined as three-dimensional (3D) cross-linked polymer networks that are swollen in solvents.[2,3] Networks swollen in water provide hydrogels, whereas those that swell in organic solvents yield organogels. Polymeric gels are broadly classified as physical and chemical gels. Physical gels can be prepared through the physical crosslinking of polymer chains via hydrophobic effects and ionic or hydrogen bonds and through the formation of crystallites within the network. Physical gels commonly redissolve upon changes in environmental conditions such as temperature, ionic strength, and pH. In contrast, chemical gels form by crosslinking through covalent bonds, leading to irreversibly insoluble networks.[4]

Cryotropic gelation has been used to prepare physically cross-linked gels by freezing and subsequently thawing aqueous solutions of poly(vinyl alcohol) (PVA).[5] PVA cryogels form via hydrogen bonds between PVA chains in the unfrozen phase, leading to microcrystalline domains that act as crosslinks.[6] PVA cryogels can be prepared from water or dimethyl sulfoxide (DMSO);[7] with DMSO as the solvent, transparent gels formed, whereas opaque PVA cryogels were formed from aqueous solutions.[8] The properties of PVA cryogels depended on multiple factors, including the molecular weight and concentration of PVA in the initial solution, additives, the solvent from
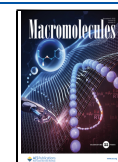
which they were formed, the cryogenic conditions (temperature and time of freezing, rates of thawing), and the number of freezing and thawing cycles.[7,9]

Physically cross-linked cryogels with antimicrobial properties and surface charge control would be beneficial for biomedical, wound-healing, and pharmaceutical applications. Due to the nonionic nature of PVA, its physically cross-linked cryogels do not exhibit antimicrobial properties. Therefore, physical cryogels based on cationic polymers are of interest for antimicrobial applications.

Polyethyleneimine (PEI) is a cationic polymer consisting of two carbon aliphatic ($-CH_2CH_2-$) spacer groups and primary, secondary, and tertiary amine groups in each repeating unit. PEI is either linear or branched, which differ in structure and some properties.[10] In linear PEI, all amine groups are secondary, whereas branched PEI has primary, secondary, and tertiary amine groups. Branched PEI is usually synthesized by ring-opening polymerization of aziridine, and L-PEI can be prepared by hydrolysis of poly(2-ethyl-2-oxazoline) under acidic or basic conditions to eliminate all of the amide groups in the side chains.[11,12] Branched PEI is an amorphous polymer that is readily soluble in water,[13] whereas L-PEI is semicrystalline and only dissolves in water at high temperatures.[13,14] Chantani et al.[14] demonstrated that temperatures >60 °C lead to increased chain mobility of L-PEI, resulting in the melting of crystallites and eventual dissolution in water.[15] PEI has a wide range of applications, including gene delivery,[16] pharmaceutical,[10] antimicrobial,[17,18] and environmental applications.[19] Branched PEI cannot be used to prepare physically cross-linked hydrogels via cryogelation techniques, but it may form hydrogels through covalent crosslinking with various reagents, such as epichlorohydrin, diglycidyl ethers of glycols, and glutaraldehyde.[20,21] In contrast, L-PEI is capable of forming physically cross-linked hydrogels, as shown by Yuan et al.[22] They reported gelation of L-PEI in water by cooling its hot aqueous solutions to room temperature, forming physical or thermoreversible gels.[22] This work provided new opportunities for the development of L-PEI hydrogels as a potential candidate for biorelated applications such as scaffolds for cell culture and controlled drug delivery.[22]

To the best of our knowledge, there are no reports in the literature on L-PEI cryogels formed by freezing and subsequent thawing of its aqueous solutions. Thus, the fabrication of physically cross-linked cryogels based on L-PEI via cryotropic gelation has advantages, since this method does not require the use of cross-linkers or initiators. Therefore, the purpose of this study was to develop L-PEI as physically cross-linked cryogels using a freeze-thawing technique and to study the influence of cooling temperature and solvent composition on the thermal, crystallinity, and rheological properties of these systems.

2. EXPERIMENTAL SECTION

2.1. Materials. Poly(2-ethyl-2-oxazoline) (50 kDa), deuterated methanol (CD_3OD), sodium hydroxide, hydrochloric acid, and ethanol were purchased from Merck (Gillingham, U.K.). All other chemicals were of analytical grade and were used without further purification.

2.2. Synthesis of L-PEI. L-PEI was synthesized by hydrolysis of poly(2-ethyl-2-oxazoline) as described by Shan et al.[23] Briefly, 10 g of poly(2-ethyl-2-oxazolines) (PEOZ) was dissolved in 100 mL of 18.0% (w/w) hydrochloric acid and then refluxed at 100 °C for 14 h to remove all amide groups in the side chains. The PEI solution obtained in hydrochloric acid was then diluted with cold deionized water (500 mL). Cold aqueous sodium hydroxide (4 M) was added dropwise to the suspension until the polymer dissolved, but with further addition of 4 M sodium hydroxide, the base form of L-PEI precipitated at pH 10−11.[11]

The precipitate was recovered by filtration, washed with deionized water, and reprecipitated twice before drying under vacuum to obtain L-PEI as a white power (yielding 3.8 g (88.4%)).

2.3. General Method to Prepare Cryogels from L-PEI. L-PEI cryogels (5% w/w) were prepared by a freezing and thawing method. For each cryogel, 100 mg of L-PEI powder was dispersed in 2 mL of deionized water. All samples were then heated at 80 °C until clear solutions were formed. The L-PEI solutions were then frozen for 3 h before being placed in an incubator at 25 °C for 24 h to thaw. As a reversibility control, the cryogels were reheated at 80 °C until they formed a clear solution. The cryogels and reversible control were characterized using X-ray diffraction. All samples were prepared in triplicate.

2.4. Effect of Thawing Temperature on the Formation of L-PEI Cryogels. L-PEI cryogels (5% w/w) were prepared as above and were all frozen at −80 °C for 3 h. The samples were then allowed to thaw at various temperatures between 20 and 80 °C for 24 h.

2.5. Effect of Different Freezing Temperatures on the Physicochemical Properties of L-PEI Cryogels. Cryogel solutions were prepared as mentioned above and then cooled to various temperatures (−196, −80, −30, and 0 °C) for 3 h, followed by thawing in an incubator at 25 °C for 24 h. Liquid nitrogen (−196 °C) and controlled temperature freezers at −80 and −30 °C were used for freezing. In addition, L-PEI solution was placed in normal ice to cool to 0 °C.

2.6. Effect of Solvent on the Formation and Physicochemical Properties of L-PEI Cryogels. First, 100 mg of L-PEI was added to 2 mL of ethanol/water mixtures. Ethanol/water mixtures that generated suspensions were heated at 80 °C in a water bath to form clear solutions, whereas samples in which L-PEI readily dissolved were prepared using a vortex mixer at room temperature. The solutions were then cooled at −80 °C for 3 h, followed by thawing in an incubator at 25 °C overnight. The rheological properties of the cryogels were characterized using a Brookfield rheometer (DV II, Germany). The samples of all cryogels were dried to a constant weight in a vacuum oven at 40 °C and then characterized by Fourier-transform infrared (FTIR) spectroscopy and X-ray diffractometry. All samples were investigated in triplicate.

2.7. ¹H Nuclear Magnetic Resonance Spectroscopy (¹H NMR). After dissolving 20 mg of dried PEOZ and L-PEI in methanol-d_4, ¹H NMR data were collected using a 400 MHz Ultrashield Plus B-ACS 60 spectrometer (Bruker, U.K.). Spectral analysis was conducted using MestReNova software.

2.8. Fourier Transformed Infrared (FTIR) Spectroscopy. Solid samples of PEOZ, L-PEI, and dried L-PEI cryogels were scanned from 4000 to 400 cm⁻¹ at 4 cm⁻¹ resolution using a Nicolet iS5-iD5 ATR FTIR spectrometer (Thermo Scientific, U.K.). Data were processed based on the average of six scans per spectrum.

2.9. Rheology. The rheological properties of L-PEI cryogels were evaluated using a strain-controlled rheometer (TA Instruments) fitted with a plate−plate geometry (25 mm diameter). The storage modulus (G'), loss modulus (G''), and complex viscosity (η^*) were recorded as a function of temperature from 20 to 80 °C for gel−sol transition experiments. The η^* was calculated according to eqs 1 and 2. In addition, the oscillation frequency mode was used to measure G', G'', and complex viscosity (η^*) at 25 °C for cryogels prepared at different cooling temperatures. The following conditions were used in these experiments: a strain of 0.5%, a gap of 0.5 mm, a constant frequency of $\omega = 6.28$ rad/s to establish the linear viscoelastic region, and a frequency sweep in the range of 0.01−100 Hz.[24]

$$\eta^* = G^*/\omega \tag{1}$$

$$G^* = \sqrt{G'^2 + G''^2} \tag{2}$$

where η^* is the complex viscosity, which is a measure of the total resistance to flow as a function of angular frequency (ω) given by the quotient of the maximum stress amplitude and maximum strain rate amplitude. G^* is the complex shear modulus that allows the viscous and elastic components to contribute to the total material stiffness.

Figure 1. Solution−gel−solution transition of L-PEI cryogels. The solution is initially heated to 80 °C, forming a transparent solution. After freezing at −80 °C for 3 h and then thawing at room temperature, an opaque gel is obtained, which, on subsequent heating to 80 °C, returns to a transparent solution (a). Appearance of L-PEI cryogels (b).
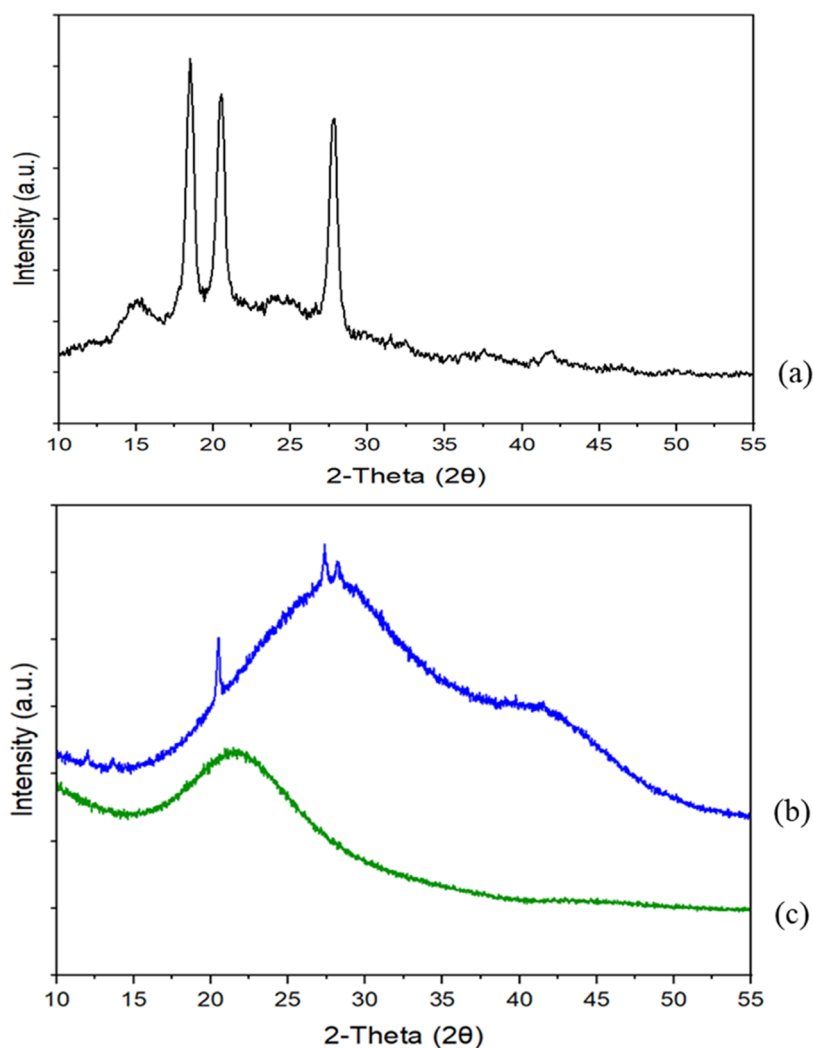

Figure 2. X-ray diffraction patterns for dry L-PEI (a) and wet cryogels prepared from L-PEI solution (b) and this sample after heating (to solution) at 80 °C (c).

The viscoelastic behavior of samples was quantified through dynamic measurements in terms of relative phase angle (tan δ = G'' / G', where δ is the loss angle). If tan δ is <1 then this indicates predominantly elastic behavior and a tan δ > 1 shows predominantly viscous behavior.[25]

2.10. Differential Scanning Calorimetry (DSC). Thermal analysis of samples was performed using DSC (TA Instruments, Germany). Dried L-PEI powder (~3 mg) was loaded into pierced Tzero aluminum pans, whereas wet cryogel samples (~3 mg) were

loaded into nonpierced Tzero aluminum pans to prevent evaporation of water. The thermal behavior of each sample was investigated from 30 to 100 °C at 10 °C/min in a nitrogen atmosphere. The melting temperature (T_m) and enthalpy of samples were determined. All samples were analyzed in triplicate.

2.11. X-ray Diffraction (XRD). Wet or dried L-PEI cryogels were placed on a silica slide and analyzed in a Bruker D8 ADVANCE PXRD equipped with a LynxEye detector and monochromatic Cu Kα_1 radiation ($\lambda = 1.5406$ Å). The samples were rotated at 30 rpm, and data were collected over an angular range of 5−60° (2θ) for 1 h, with a step of 0.05° (2θ) and count time of 1.2 s. The results were analyzed using Origin software. All samples were analyzed in triplicate.

From the data, the percentage of crystallinity (χ) and crystallite size (P) were calculated according to eqs 3 and 4 as follows[26]

$$\chi = \frac{\int A_c}{\left(\int A_c + \int A_a\right)} \tag{3}$$

where A_c and A_a are the values of area under the peaks corresponding to crystalline and amorphous phases, respectively.

$$P = \frac{K\lambda}{\beta \cos\theta} \tag{4}$$

where P is the mean size of the ordered (crystalline) domains, K is 0.9 (Scherrer's constant), λ is the X-ray wavelength (0.15406 nm), β is the peak full width at half-maximum (FWHM, radians), and θ is the Bragg angle that is obtained from the peak position (radians).

2.12. Scanning Electron Microscopy (SEM). SEM was used to investigate the porous structure of L-PEI cryogels. Briefly, the freeze-dried cryogel samples were examined using a FEI Quanta 600 FEG SEM microscope (Field Electron and Ion Company) with an acceleration voltage of 20 kV. The samples were coated with gold sputter to facilitate high-resolution imaging.

2.13. Swelling Analysis. Freeze-dried L-PEI cryogels were placed into Petri dishes with 40 mL of deionized water at 25 °C. The Petri dishes were then placed on graph paper, and changes in the sizes of the dry cryogels were measured over time. The diameter of each sample was measured five times in different directions, and mean ± standard deviation values were calculated. The swelling ratio of L-PEI cryogels was calculated according to eq 5

swelling ratio = diameter of swollen gels/diameter of dry gels

$$\tag{5}$$

2.14. Statistical Analysis. The results are presented as mean ± standard deviations for no fewer than three independent experiments. Student's t-test and one-way ANOVA were used for analysis of the data to determine the extent of any differences between the cryogel preparation methods.

3. RESULTS AND DISCUSSION

3.1. Formation of Physically Cross-Linked Cryogels based on L-PEI. To prepare our initial L-PEI cryogels, the polymer was first dissolved at 80 °C in water to break crystalline domains before freezing at −80 °C, followed by thawing at room temperature (25 °C), forming stable cryogels (Figure 1). It is known that L-PEI exhibits water-induced phase transitions between four types of crystalline hydrates depending on the amount of absorbed water/anhydrate (EI monomer unit/water molecules = 1:0), hemihydrate (1:0.5), sesquihydrate (1:1.5), and dihydrate (1:2).[27] The polymer chain conformation changes from a double helix in the anhydrate to a planar-zigzag form in the hemi-, sesqui-, and dihydrate forms. In brief, the PEI double helix forms via intermolecular NH⋯N hydrogen bonds. When water molecules are absorbed into the lattice, NH⋯O and OH⋯N bonds form between zigzags of PEI chains and water molecules.[28] In this study, L-PEI cryogels in water formed a dihydrated structure due to the high absorption of water
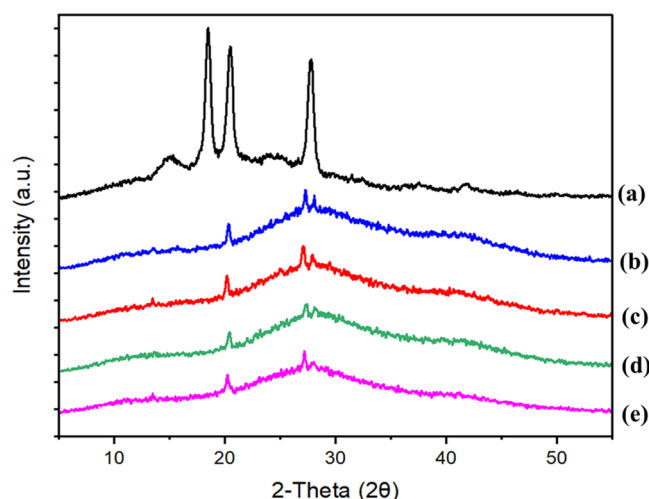

Figure 3. X-ray diffraction spectra of dry L-PEI (a) and wet L-PEI cryogels prepared at various freezing temperatures: −196 °C (b), −80 °C (c), −30 °C (d), and 0 °C (e).

Table 1. Enthalpy, Degree of Crystallinity, and Mean Crystal Size of L-PEI Cryogels Prepared at Different Freezing Temperatures

freezing temperature (°C)	enthalpy (ΔH, J/g)[a]	degree of crystallinity (%)[b]	mean crystal size (nm)[b]
dry L-PEI	94.2 ± 14.9	30.8 ± 0.39	3.50 ± 0.03
−196	10.7 ± 0.2	1.65 ± 0.10	0.73 ± 0.07
−80	9.8 ± 0.8	1.51 ± 0.18	0.76 ± 0.03
−30	9.2 ± 0.1	1.17 ± 0.22	0.78 ± 0.02
0	7.7 ± 1.7	1.00 ± 0.14	0.83 ± 0.08

[a]Determined from DSC experiments. [b]Determined from XRD experiments.
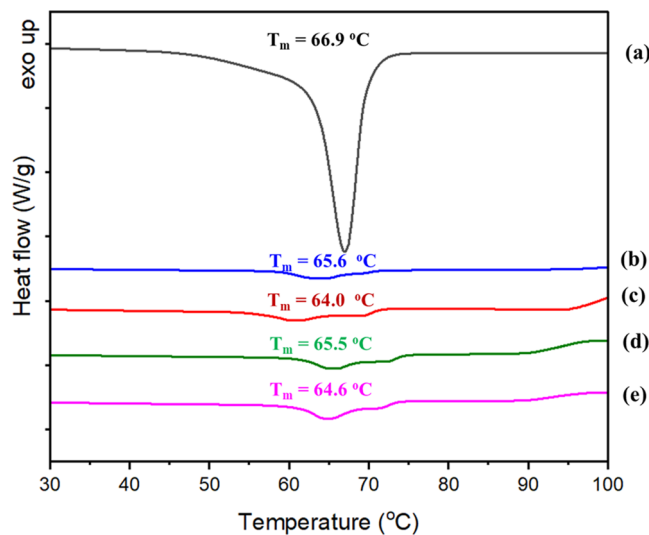

Figure 4. DSC thermograms of dry L-PEI (a) and wet L-PEI cryogels prepared at various freezing temperatures: −196 °C (b), −80 °C (c), −30 °C (d), and 0 °C (e).

molecules into L-PEI lattices (determined by XRD, see below). In addition, thermal cycling promotes physical crosslinking via the formation of structured crystalline domains of the polymer chains through phase separation. Several phases form during the thermal cycling process. Initially, as the gel cools down to low
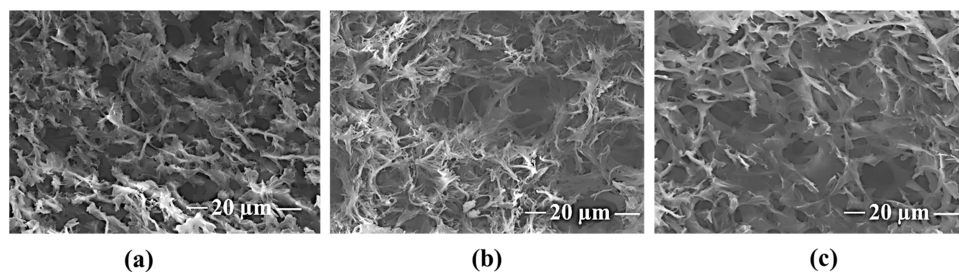
Figure 5. SEM images of freeze-dried L-PEI cryogels prepared at various freezing temperatures: −196 °C (a), −80 °C (b), and 0 °C (c).
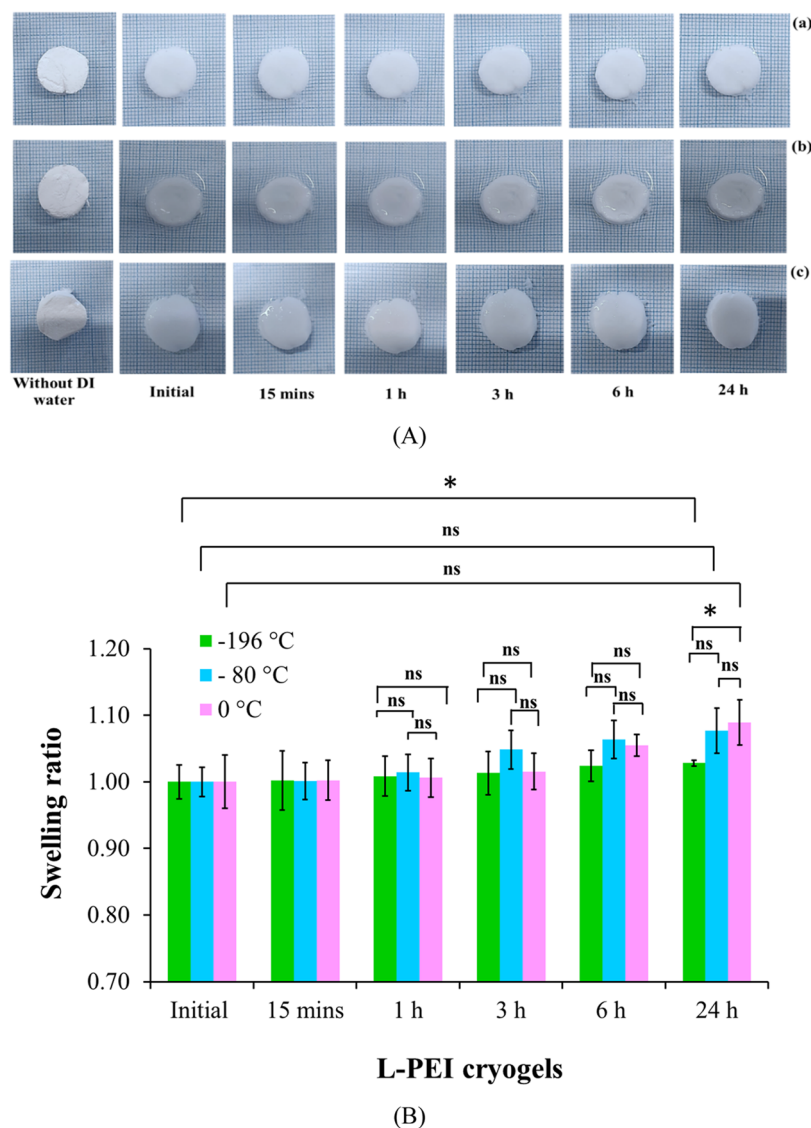
Figure 6. Swelling of freeze-dried L-PEI cryogels prepared at different freezing temperatures: −196 °C (a), −80 °C (b), and 0 °C (c) in deionized water: physical appearance (A) and swelling ratios (B). Statistically significant differences are given as *; $p < 0.05$: ns, no significance.

temperatures, the water phase freezes. This creates regions of high polymer concentration, which can thus form crystallites and regions of low polymer concentration, resulting in pore formation. The frozen gel was then thawed at room temperature to obtain interconnected macroporous gels.[29] While the formation of physically cross-linked cryogels from L-PEI using the freezing and thawing method has not been previously reported, it is likely that the mechanisms are similar to those in PVA cryogels.[30] Lozinsky et al.[31] found that PVA cryogel rigidity

increased with increasing concentrations of PVA due to the increase in hydroxyl groups for intermolecular hydrogen bonds. Hassan and Peppas[32] reported that increasing the concentration of PVA resulted in a highly stable gel due to the high degree of crystallinity and lower secondary crystallization. Caló et al.[8] reported poly(vinyl alcohol)-Gantrez AN cryogels formed by a freezing and thawing technique. Inter- and intrachain hydrogen bonding formed more rapidly in the unfrozen liquid microphases where the reagents were more highly concentrated by the
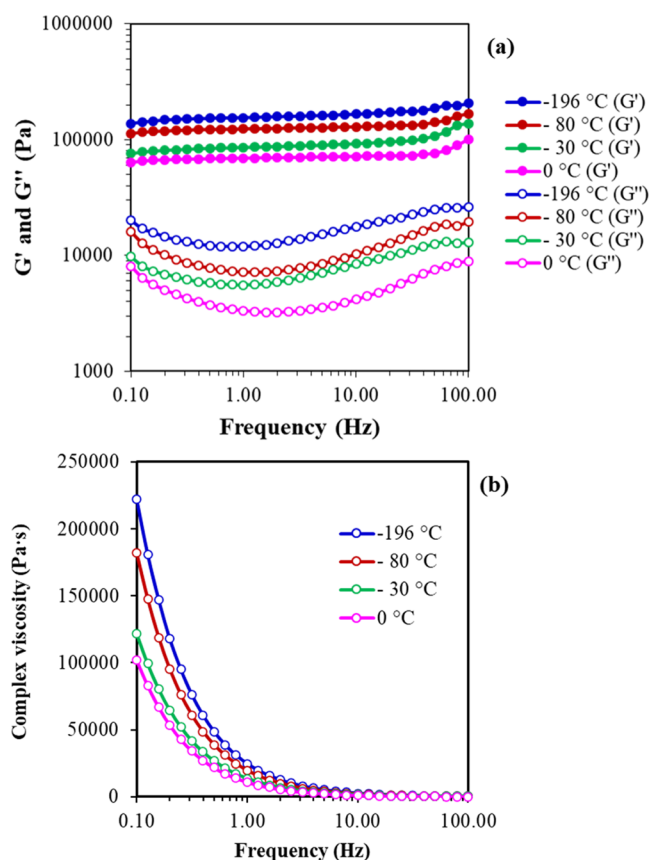
Figure 7. Storage (G') and loss (G'') modulus (a) and complex viscosity (η^*, b) of L-PEI cryogels prepared at different freezing temperatures.

formation and expansion of solvent crystals during freezing. Subsequent thawing resulted in a network of interconnected pores within the cryogel internal structure, which influenced the physical and mechanical properties of the samples.

The reversibility of our L-PEI cryogels, shown by reheating the gel at 80 °C, which formed a transparent solution, confirms their physical rather than chemical crosslinking (Figure 1).

Evidence for crystalline domains was obtained by X-ray diffractometry. The L-PEI starting material showed diffraction peaks at $2\theta = 18$, 20, and 28°, in agreement with literature reports for the hemihydrated crystalline structure of PEI.[22] The diffraction peaks for cryogels appeared at $2\theta = 20$, 27, and 28° (Figure 2),[16,19] typical for the dihydrate crystalline state.[16,19] The formation of physical cryogels based on L-PEI is caused by the change in the polymer chain conformation from a double helix in the anhydrate to a planar-zigzag conformation as water molecules are absorbed into the L-PEI lattice and by the formation of NH···O and OH···N bonds between the zigzag PEI chains and water molecules.[28] Further, when the cryogels were reheated at 80 °C, again forming a clear solution, the diffractogram showed no evidence of crystalline domains due to their melting caused by the breakdown of hydrogen bonds at high temperatures. Yuan et al.[22] reported the gelation of L-PEI following the heating of L-PEI solutions at 80 °C and then cooling down to room temperature. Again, gel formation was attributed to the crystallization of L-PEI, and they showed that the L-PEI used to prepare gels was semicrystalline, whereas their 5% L-PEI hydrogel exhibited the same diffraction pattern as our cryogels, with PEI in a dihydrate crystalline state.
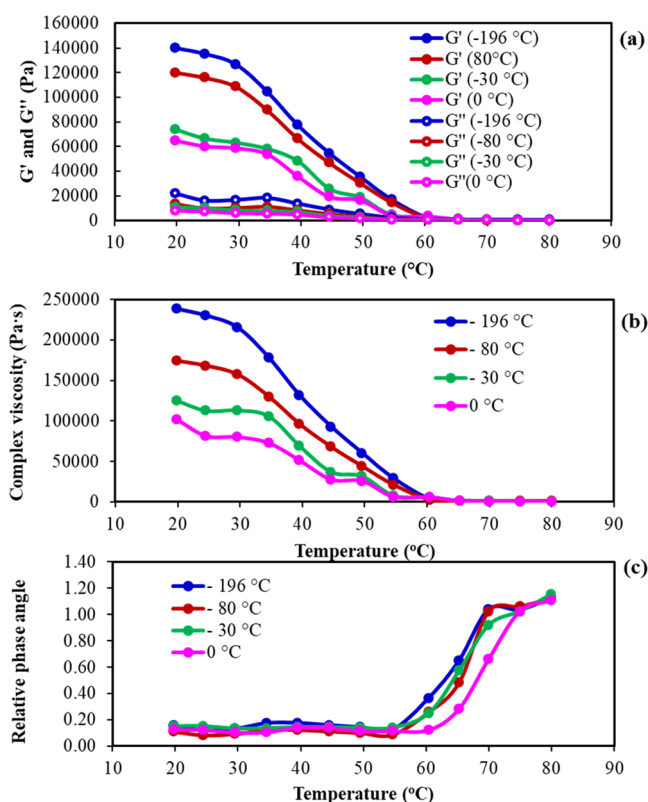

Figure 8. Storage (G') and loss (G'') modulus (a), complex viscosity (η^*, b), and relative phase angle (c) of L-PEI cryogels prepared at different freezing temperatures as a function of temperature.
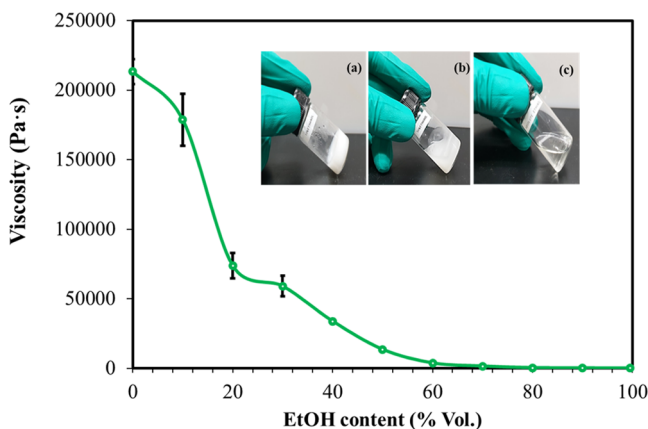

Figure 9. Viscosities of L-PEI samples following their freezing and thawing in different ethanol/water mixtures and images of samples prepared at 20% (a), 50% (b), and 100% (c) EtOH.

3.2. Effect of Different Freezing Temperatures on the Thermal, Crystallinity, and Rheological Properties of Cryogels based on L-PEI. Given the role of phase separation in cryogel formation, we anticipated that the size of ice crystals formed during freezing L-PEI solutions would affect the mechanical and physical properties of the cryogels. Specifically, the rate and extent of freezing could affect both PEI crystallization and the pore size resulting from ice crystals. Ceylan and Okay[33] previously reported the effect of freezing temperature on the properties of gels prepared by solution crosslinking of butyl rubber in frozen benzene solutions using sulfur monochloride as a crosslinking agent. Therefore, the effect
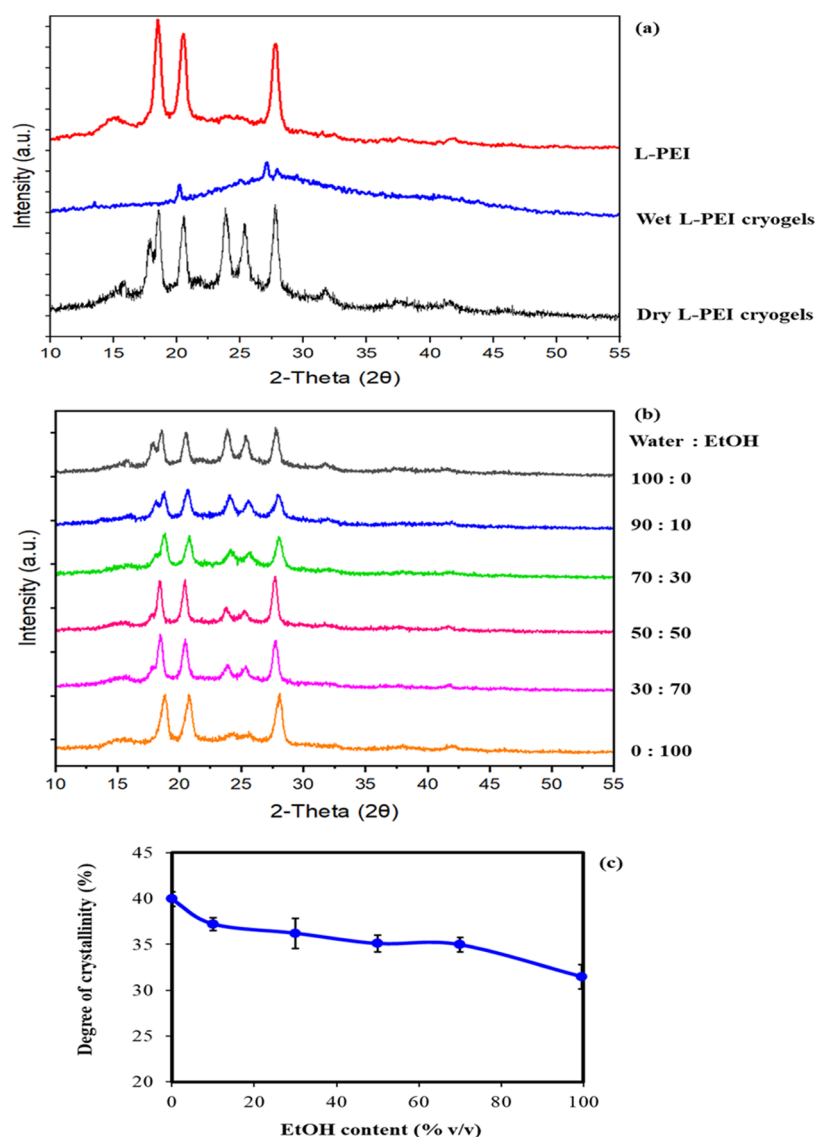
Figure 10. XRD spectra of L-PEI, wet L-PEI, and dried to constant weight L-PEI cryogels formed from water (a); XRD spectra (b); and degree of crystallinity (c) of dried L-PEI cryogels prepared from different ethanol/water mixtures. The sample prepared from 100% ethanol did not form a gel but was dried and resulted in the same diffraction pattern as the starting L-PEI (as in a).

of freezing conditions on the physical properties of L-PEI cryogels was evaluated in this work.

Figure 3 shows X-ray diffractograms of dry L-PEI and L-PEI cryogels produced when polymer solutions were frozen at −196, −80, −30, and 0 °C. The XRD patterns of all cryogels were in agreement with the above results, giving peaks at $2\theta = 20, 27,$ and 28° typical for the dihydrate crystalline form ($EI/H_2O =$ 1:2).

The degree of crystallinity of L-PEI in cryogels can be estimated using the area under the sharp (crystalline) peaks over the total area and was clearly dependent on the freezing temperature; crystallinity within the samples increased as the freezing temperature decreased, with over 1.6% crystallinity seen when the solutions were rapidly frozen at −196 °C. (Table 1). As expected, the size of the crystallites also reduced when frozen more rapidly since molecular mobility is hindered at lower temperatures. This inhibits crystallite growth, resulting in a greater number of smaller crystallites when frozen rapidly to −196 °C compared to the larger but fewer crystallites when frozen to 0 °C. These results are in good agreement with other

studies.[28,29] A lower freezing temperature contributed to smaller cryogel crystals and a smaller cross-section of macropores within the gel.[34]

Figure 4 shows DSC thermograms of L-PEI and L-PEI cryogels produced when frozen at −196, −80, −30, and 0 °C, respectively. It is known that dry L-PEI exhibits a sharp endothermic transition at 66.9 °C, which corresponds to the melting of its crystalline domains, and the melting temperature of L-PEI correlated to a report of Shan et al.[23] All thermograms of L-PEI cryogels also show this endothermic event around this temperature, but the peaks are broad and potentially show two overlapping events, one from the melting of crystalline domains as above and a second thermal event with an earlier onset possibly due to thermal dissociation of inter-macromolecular hydrogen bonds. The area under the endothermic peak was calculated to provide the enthalpy of these processes (Table 1). The enthalpy of L-PEI melting was 94.2 J/g, whereas L-PEI cryogels exhibited the highest enthalpy values when frozen at −196 °C, followed by −80, −30, and 0 °C with a linear

correlation ($R^2 = 0.9035$) between the enthalpy values and the XRD-determined degree of crystallinity (Figure S3).

The porous structure of freeze-dried L-PEI cryogel samples generated at various freezing temperatures was investigated by scanning electron microscopy (SEM). Figure 5 shows SEM images of selected cryogel samples. The results indicate that all L-PEI cryogels have a macroporous structure with highly interconnected pores. Unfortunately, due to the irregular shape of the pores, the average pore size could not be determined. However, these images indicate that the freezing temperatures do not radically change the porosity of the samples.

Initially, an attempt was made to study the swelling properties of freeze-dried cryogels using a gravimetric technique. However, once immersed in deionized water, the cryogels rapidly lost their integrity and disintegrated during sample handling. The swelling and mechanical properties of these samples are very different from those of the cryogels based on PVA-Gantrez AN, reported by Caló and co-workers.[8] L-PEI cryogels are more fragile and can easily disintegrate upon swelling, whereas PVA-Gantrez AN cryogels are highly elastic and are achieved high swelling ratios. Therefore, a less-invasive approach was subsequently used with a visual inspection of changes to L-PEI cryogel volumes. Figure 6 shows the changes in the appearance and swelling ratio of dry L-PEI cryogels prepared at different freezing temperatures during swelling in deionized water. In general, swelling ratios were modest, with no significant changes over the first 6 h for any given cryogel or between cryogels prepared at different freezing temperatures. After 24 h, the material prepared by freezing at 0 °C gave a significantly greater swelling ratio than that prepared at −196 °C ($p < 0.05$), and this sample, produced at the lowest temperature, had increased swelling at 24 h compared to its initial size. These modest changes are consistent with the SEM data: the similarity in sample porosity results in similar swelling behavior.

The rheological behavior of L-PEI cryogels prepared at the different freezing temperatures was studied in an oscillation frequency mode at 0.1−100 Hz with a fixed strain of 0.5% (Figure 7). The results demonstrated that the freezing temperature affected the elastic behavior of L-PEI cryogels. The storage (or elastic) modulus values (G') of all of the samples were greater than the loss (also known as the viscous) modulus (G'') over the whole frequency range, demonstrating predominantly gel-like rheological behavior. The storage modulus has been used to predict the stiffness of a material,[29,30] and so the greater G' values seen with samples produced at lower freezing temperatures indicate increased mechanical strength. Likewise, the loss modulus (G'') generally increased as the freezing temperature decreased. However, between 0.1 and ~1 Hz, the loss modulus decreased for all samples and then increased again with increasing oscillation frequency up to 100 Hz due to a reduction in the intermolecular hydrogen bonding as the cryogels were disrupted. Consequently, the complex viscosity (η^*) decreased as the oscillation frequency increased from 0.1 to 100 Hz, which is consistent with the reduction in the intermolecular hydrogen bonding as a result of disruption of the cryogels. Our rheological data correlates well with that from X-ray diffraction and thermal analysis. Comparison of the degrees of crystallinity values with the rheological properties of the samples indicates that mechanically stronger gels are more crystalline; the crystallites act as physical crosslinking junctions and also smaller water crystals formed at lower freezing temperatures, resulting in smaller pores in the cryogels. Lozinsky[35] also reported that preparing physically cross-linked

polysaccharide cryogels at lower freezing temperatures resulted in increased gel strength.

The storage modulus (G'), the loss modulus (G''), and complex viscosity (η^*) of L-PEI cryogels were investigated as a function of temperature from 20 to 80 °C. Figure 8 shows the effect of temperature on the rheological properties of L-PEI cryogels prepared at different freezing temperatures. As expected, both the storage and loss modulus values as well as shear viscosity decrease with increasing temperature. Interestingly, the G', G'', and η^* data suggest that the cryogels are essentially liquid at 60 °C. Our thermal analysis (Figure 4) showed that L-PEI melted at ~67 °C, although the onset for the melt was earlier. Thus, it may be that the gel−sol transition seen by rheology is predominantly driven by disruption of the inter-macromolecular hydrogen bonds before the melting of the polymer crystalline domains. To explore this further, the relative phase angle (tan $\delta = G''/ G'$, where δ was the loss angle) was calculated (Figure 8c). When tan δ is lower than 1, then this indicates predominantly gel behavior, whereas values higher than 1 reflect viscous or liquid behavior.[25] The data show a gradual increase in the phase angle between 60 and 70 °C and only exceeds a value of 1 beyond the melting temperature of L-PEI. This indicates that while the gel structure is initially lost by disruption of the intermolecular hydrogen bonds, the cryogels retained some structural organization from the crystalline domains until melting, where the systems were truly liquid. However, the rheological data demonstrate that the hydrogen bonds are predominantly responsible for the structural integrity of the cryogels.

3.3. Effect of Solvent Composition on Formation, Crystallinity, and Rheological Properties of Cryogels based on L-PEI. The influence of solvent composition on the formation and physicochemical properties of L-PEI cryogels was explored. L-PEI was dissolved in ethanol/water mixtures before freezing at −80 °C for 3 h and subsequently thawing at 25 °C. Dissolving L-PEI in ethanol alone did not result in the formation of cryogels; our samples were cooled down to −80 °C, whereas the freezing point of ethanol is −115 °C (Table S2). However, L-PEI in mixtures of water with ethanol did form gels. With increasing ethanol content, the gels became softer, probably due to reduced intermolecular hydrogen bonding between L-PEI macromolecules because of competition with solvent molecules (Figure 9). The transition from hard to soft gels was associated with a decrease in gel viscosity as the concentration of ethanol in the solvent mixtures increased. These results also correspond with literature reports that reported that increasing concentrations of alcohol in aqueous mixtures decreased both the rigidity and fusion temperature of PVA cryogels.[9]

As described above, L-PEI starting material was shown to be a hemihydrate, which then reverted to a dihydrate structure when formed into a hydrogel from water. When dried to constant weight, these samples transitioned to a sesquihydrate (EI/H_2O = 1:1.5), with further diffraction peaks at $2\theta = 24$ and $25°$ (Figure 10a). These findings are consistent with data from Hashida and Tashiro,[36] who demonstrated that PEI exhibits water-induced phase transitions between four crystalline hydrates: anhydrate (El/water = 1:0), hemihydrate (1:0.5), sesquihydrate (1:1.5), and dihydrate (1:2). From the diffraction patterns, the L-PEI and dried L-PEI cryogels were estimated to have a crystallinity of 31 and 40%, respectively (Table S3), similar to that reported by Lázaro-Martinez et al.[37] The increased crystallinity of dried L-PEI cryogels when compared to L-PEI

may result from the intermolecular hydrogen bonding between NH⋯O and OH⋯N in L-PEI molecules.

Figure 10b shows the XRD patterns of dried (to constant weight) L-PEI cryogels prepared in various ethanol/water mixtures, with significant peaks at $2\theta = 20, 24, 25, 27,$ and $28°$. The sample from 100% ethanol did not form a cryogel but was dried to a constant weight, giving a diffraction pattern consistent with the starting L-PEI (as a hemihydrate). The diffraction peaks in the dried hydrogels are in agreement with the typical pattern of sesquihydrates, consistent with the literature[36] and with the pattern resulting from the dried cryogel produced from water alone. The degree of crystallinity of L-PEI in the dried cryogels tended to decrease ($p < 0.05$) as the concentration of ethanol in L-PEI ethanol/water mixtures increased and was significant ($p < 0.05$) when compared with the gel produced from water (39.9% crystalline) and when recovered from ethanol alone (31.5%) (Figure 10c). It is feasible that ethanol either competes with or interferes with the intermolecular hydrogen bonding between NH⋯O and OH⋯N in L-PEI.

4. CONCLUSIONS

Cryogels based on L-PEI were formed by the freeze/thaw process of concentrated L-PEI solutions in water. The physically cross-linked cryogels were also validated by demonstrating that they formed a clear solution upon heating to 80 °C due to the melting of the L-PEI crystalline domains. L-PEI cryogels were found to have a higher enthalpy, crystallinity, and strength when cooled down to temperatures below 0 °C. Additionally, increasing ethanol content in water/ethanol mixtures used to prepare L-PEI cryogels resulted in a decrease in mechanical strength, crystallinity, and viscosity. This could be because the intermolecular hydrogen bonds between NH⋯O and OH⋯N in L-PEI decreased, most likely due to hydrogen bond competition from the solvent molecules. This study demonstrates that the physical properties of L-PEI cryogels can be manipulated by controlling the cooling rate and solvent composition used to prepare the cryogels. Hence, due to the unique chemical and physical properties of L-PEI, these novel physical cryogels have potential biomedical and antimicrobial applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.macromol.2c01308.

> ^{1}H NMR spectra of PEOZ and L-PEI in methanol-d4; FTIR spectra of PEOZ and L-PEI; correlation between enthalpy (J/g) and degree of crystallinity (%); effect of thawing temperatures on the formation of L-PEI cryogels; effect of temperature on the rheological behavior of L-PEI cryogels; FTIR spectra of dried L-PEI cryogels with different alcohol/water mixtures; FTIR peaks of dried L-PEI cryogels with different alcohol/water mixtures; freezing point of water/ethanol mixture solvent, and degree of crystallinity and the average size of crystals of L-PEI and dried L-PEI cryogels (PDF)

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Author Contributions

S.S.: methodology, investigation, validation, and writing original draft; A.C.W.: writing—review & editing and supervision; V.V.K.: conceptualization, writing—review & editing and supervision.

Notes

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