JOURNAL OF Applied Polymer SCIENCE

Poly ionic liquid cryogel of polyethyleneimine: Synthesis, characterization, and testing in absorption studies

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ABSTRACT: Here, we report the synthesis of polyethyleneimine (PEI) cryogels for the first time via cryopolymerization technique. The crosslinking of amine groups on the branched PEI chains is accomplished with epoxy groups of glycerol diglycidyl ether (GDE) based on epoxy–amine reactions in excess water at −18 °C in about 16 h. Superporous PEI cryogels with pore sizes >100 μm were shown to have very fast equilibrium swelling behavior, e.g., 10 s to reach maximum swelling in DI water. Furthermore, the synthesized PEI cryogels were exposed to anion exchange reaction after protonation by HCl treatments to generate PEI ionic liquid cryogels containing hexafluorophosphate, thiocyanate, dicyanamide, and tetrafluoroborate. It was also demonstrated that PEI cryogels modified with $[PF_6]^-$ absorbed 47.8 ± 5.7 mg/g of bovine serum albumin (BSA). Moreover, PEI cryogels were shown to be very useful as simple filtration filling materials for the direct removal of organic dyes such as methyl orange (MO) and eosin Y (EY) from their corresponding aqueous solutions with 98.5 and 98.6% yields, respectively. The separation of methylene blue (MB) from MO and EY mixture by using PEI cryogels as column filler materials was also demonstrated. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43478.

KEYWORDS: adsorption; gels; ionic liquids; porous materials; separation techniques

Received 10 November 2015; accepted 25 January 2016
DOI: 10.1002/app.43478

INTRODUCTION

Cationic polymers are very attractive for numerous applications, such as drug delivery systems,[1,2] gene delivery,[3,4] environmental applications,[5,6] and so on. Amine-based cationic polymers, especially, are very resourceful due to their modifiable nature.[7–9] Introduction of new functional groups to the amine groups provides additional properties such as new functional groups, surface charge control, and tunable hydrophilic/hydrophobic characteristics.[7,8] Therefore, many researchers have paid attention to amine-based polymers, such as polyethyleneimine (PEI),[10–14] chitosan,[15–19] melamine,[20–22] and so on. In recent years, among the amine-based polymers PEI has drawn special attention due to its superior properties for DNA transfection[23–25] and CO_2 capture.[26] PEI possesses the highest DNA transfection capability[27,28] due to its relatively efficient ability to complex with higher amounts of DNA.[4] In recent years, materials derived from PEI have been investigated for CO_2 absorption.[29,30] For example, materials such as zeolites,[31] oxides,[32] metal-organic frameworks,[33] and amine-containing materials[34] have been generally investigated for CO_2 adsorption or capture. Because of the high amine content of PEI, materials derived from PEI are very effective for CO_2 adsorption.[26,29,30] Moreover, due to the cationic nature and tunable charge properties of PEI, it is possible to prepare PEI-based ionic liquid microgels.[7,8] Polymer-based ionic liquids (PILs) can be synthesized by two main paths[35]; polymerization of ionic liquid monomers,[36] and anion exchange reaction of existing polymers.[8] For the preparation of PIL from cationic polymers, hexafluorophosphate ($[PF_6]^-$), thiocyanate ($[^-SCN]$), dicyanamide ($[^-N(CN)_2]$), and tetrafluoroborate ($[BF_4]^-$) anions have been widely used in the anion exchange procedure with anions of cationic polymer.[8,35,37] The generation of PILs instead of ionic liquids provides great advantages for spatial controllability, mechanical stability, processability and durability,[38] boosting the applications for ionic liquids by combining polymer properties with ionic liquid properties, e.g., room temperature ionic liquids. If a crosslinked network is generated, PIL may be called PEI hydrogels, and special class of hydrogels which are cryogels synthesized under cryogenic conditions (below the freezing point of solvent). As the solvent is water for hydrogels, under cryogenic conditions,

Additional Supporting Information may be found in the online version of this article.

the ice crystals generate a superporous network because the polymerization and crosslinking takes place around the ice crystals formed under cryogenic conditions. Upon thawing, the ice templates melt and pore sizes of about the size of the ice crystals, ranging from few tens micrometer and few hundreds of micrometer, are generated and hydrogels are called superporous polymeric networks.[39] The major advantages of cryogels are high porosity, structural flexibility, high mechanical strength, and fast responsiveness in comparison to common hydrogels.[40,41]

In this study, we report the synthesis of PEI cryogels from branched PEI of different molecular weight aqueous solutions by using an epoxy–amine reaction under cryogenic conditions. The synthesized PEI cryogels were characterized in terms of swelling %, densities (dry and swollen), pore volume %, porosity % values, and mechanical strength. PEI cryogels were characterized by using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectrophotometer, and thermogravimetric analyzer (TGA). Furthermore, prepared PEI cryogels were treated with HCl for preparation of poly ionic liquids through an anion exchange reaction using ammonium hexafluorophosphate, potassium thiocyanate, sodium dicyanamide, and sodium tetrafluoroborate as anion sources. Furthermore, the PEI cryogels were demonstrated to be very useful as absorbing materials for biological proteins, such as BSA, and as column filler materials for removal organic dyes such EY, MO and methyl, and for separation of MB from organic dye mixtures.

EXPERIMENTAL

Materials

PEI (PEI, 50 wt % soln. in water, M_n: 1200, M_n: 1800, and M_n: 60,000, d: 1.08) was purchased from Sigma–Aldrich. Glycerol diglycidyl ether (GDE, $\leq$100%, Sigma Aldrich) was used as a crosslinker. Sodium hydroxide (NaOH, 98–100.5%, Sigma Aldrich) and hydrochloric acid (HCl, 36.5–38%, Sigma Aldrich) were used as modifying agents. Besides, ammonium hexafluorophosphate (AHFP, 99%, Aldrich), potassium thiocyanate (KSCN, 99%, Merck), sodium dicyanamide (SDCA, 96%, Aldrich), and sodium tetrafluoroborate (STFB, 97%, Merck) were used as anion sources for polymeric ionic liquid preparation. Eosin Y (EY, 90%, Sigma Aldrich), methyl orange (MO, Reag. Ph. Eu., Fluka), and methylene blue hydrate (MB, 97%, Sigma) were used as organic dyes. Bovine serum albumin (BSA, $\geq$96%, Sigma) was used for protein absorption studies. Distilled water (DI, 18.3 MΩ cm) was used to wash cryogels.

Synthesis of PEI Cryogel

The PEI cryogels were synthesized with epoxy–amine reaction[42–45] by cryopolymerization technique.[39–41] A certain amount of PEI solutions (1 mL, 50% in water) was placed into vials separately and brought to 10 mL volume with DI water (9 mL), vortexed for 2 min, and placed in a deep freezer for about 3 min. After that, a certain amount of crosslinker (GDE) (10% mol based on the repeating unit of PEI) was added into the solution and vortexed, and placed quickly into plastic straws (~8 mm diameter). Then, these plastic straws were placed in a −18 °C freezer for about 16 h to complete cryopolymerization. The synthesized PEI cryogels were cut into cylindrical shapes, washed with DI water five times, and dried in an oven at 50 °C.

Anion Exchange of PEI Cryogels

The synthesized PEI cryogels were exposed to anion exchange reaction using SDCA, AHFP, STFB, and KSCN as the sources of anions to include hexafluorophosphate ($(PF_6)^-$), thiocyanate ($[SCN]^-$), dicyanamide ($[N(CN)_2]^-$), and tetrafluoroborate ($[BF_4]^-$), respectively, within the PEI cryogels. In short, a certain amount of (1 g) PEI cryogels were treated with 200 mL 1*M* HCl for about 4 h at room temperature and washed three times with water. The solutions of anions were prepared in beakers separately with 1.5-fold excess of anions based on repeating unit of PEI by dissolving corresponding amounts in 50 mL of DI water. Then PEI cryogels were placed in the ionic liquid anion solution and exchange reactions were carried out for 24 h under 200 rpm mixing rate at room temperature.

Characterization of PEI Cryogels

The PEI cryogels were cut approximately to 5 mm diameter and 5 mm length and dried in an oven at 50 °C to a constant weight for swelling studies. The weight of dried PEI cryogels (m_{dry}) and swollen PEI cryogels ($m_{swollen}$) were used to calculate maximum swelling % by using eq. (1)[46]:

$$Smax\,\% = m_{swollen} \times 100 \quad (1)$$

The density of PEI cryogels was calculated with measurements of the weight, diameter and height (for volume, V) of a cryogel before and after swelling in DI water. The density of dry cryogel (d_{dry}) and swollen cryogel ($d_{swollen}$) was calculated by dividing the weight of the cryogel by its volume:

$$d = \frac{m}{v} \quad (2)$$

The pore volume (PV) was calculated using cyclohexane uptake. The weight of the dried PEI cryogels (m_{dry}) and weight of the cyclohexane uptaken by PEI cryogels ($m_{swollen}$) were calculated using eq. (3)[46]:

$$PV = m_{swollen} \times 100 \quad (3)$$

Porosity (P) of the cryogels was calculated using the weight of cryogel determined after deswelling ($M_{squeezed}$) in cyclohexane and weight of DI water swollen cryogel ($m_{swollen}$) by using eq. (4)[46]:

$$P = m_{swollen} \times 100 \quad (4)$$

All the experiments were repeated five times, and their average values are given.

The thermal behavior analysis of PEI-based cryogels were determined via TG analysis (TG/DTA 6300, SEIKO) measurements by heating up to 750 °C with 10 °C/min heating rate under N2 flow of 200 mL/min.

The SEM images of freeze-dried PEI cryogels were obtained using an SEM (JEOL JSM-5600) with an operating voltage of 20 kV. The images were acquired after placing PEI cryogels onto carbon tape-attached aluminum SEM stubs at room temperature after coating with gold to a few nanometer thicknesses under vacuum.

Protein Adsorption by PEI Cryogels

Lyophilized powder of BSA (agarose gel electrophoresis) protein with molecular weight of ~66 kDa was used for the protein adsorption studies from aqueous environments. A certain amount of PEI cryogel, 0.05 g, was placed into a vial containing

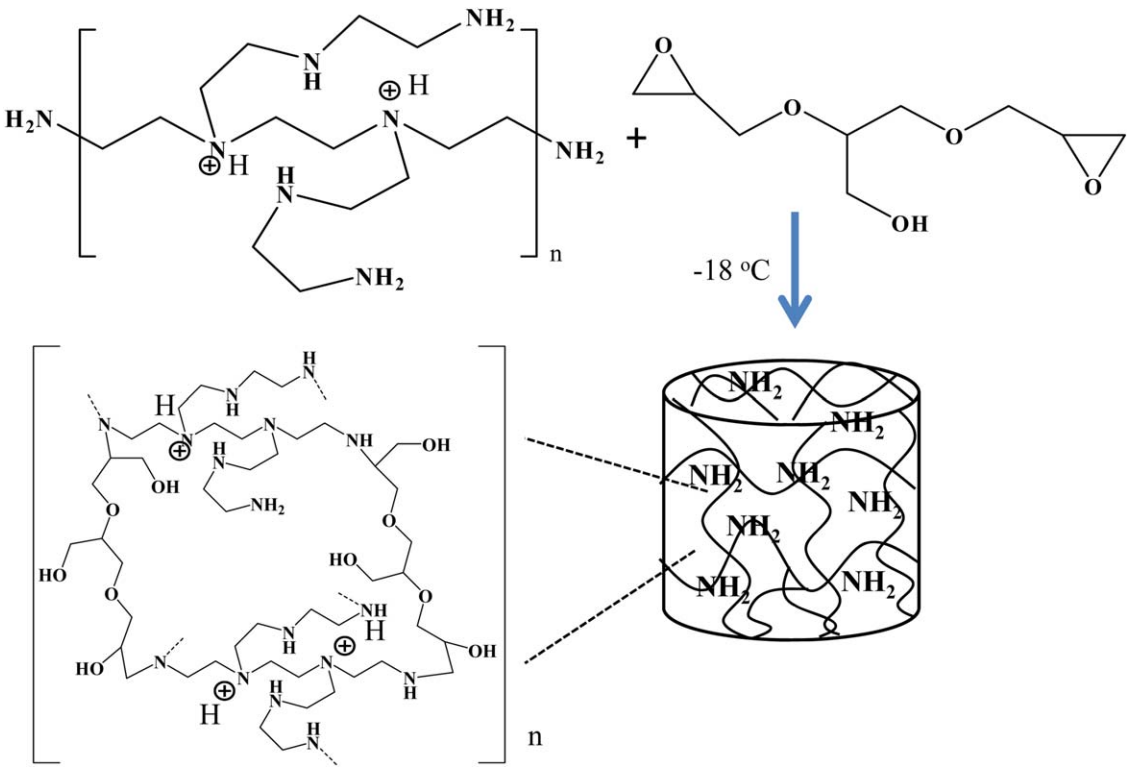

Figure 1. The schematic presentation of synthesis of glycerol diglycidyl ether (GDE) crosslinked PEI cryogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

20 mL 300 ppm BSA solution for 4 h. The amounts of BSA were determined via UV–vis spectroscopy (UV–vis, T80, PG Instruments Limited) from a previously constructed calibration curve at 280 nm that is maximum absorption wavelength for BSA in water. All the experiments were repeated three times, and the average values are reported.

Removal and Separation of Organic Dyes by PEI Cryogels

PEI cryogels were used in removal and separation of organic dyes such as EY, MO, and MB by placing PEI cryogels in glass pasteur pipettes (~0.5 cm diameter, and 15 cm height) employing them as a column filling material. For this experiment, 20 mL of $16 \times 10^{-5}M$ EY, $40 \times 10^{-5}M$ MO, and $5.2 \times 10^{-5}M$ MB solutions were used. After placing a piece of PEI cryogel (~0.5 cm diameter and 0.5 cm height under dry condition) within the pipettes, the dye solutions were passed through the column via gravity.

Removal of Eosin Y, Methyl Orange, and Methylene Blue Using PEI Cryogels. The dye solutions, e.g., 15 mL $16 \times 10^{-5}M$ EY, were poured into the column filled with PEI cryogels under free fall for purification purposes. The UV–vis spectrum of the purified liquid phase was also checked using a UV–vis spectrophotometer at their corresponding maximum absorption wavelengths, which are 514, 464, and 664 nm for EY, MO, and MB, respectively. The same procedures were followed for MO ($40 \times 10^{-5}M$) and MB ($5.2 \times 10^{-5}M$) solutions.

Separation of Methylene Blue from Methyl Orange and Methyl Orange-Eosin Y Mixture. The mixture of organic dyes was prepared by using 7.5 mL MO and 7.5 mL MB solutions (MO-MB) and 5 mL EY, 5 mL MO, and 5 mL MB solutions (EY-MO-MB) with reported concentration in Removal and Separation of Organic Dyes by PEI Cryogels section. The UV–vis spectra of these mixtures were taken before and after being placed into the column separately.

RESULTS AND DISCUSSION

Synthesis and Characterization of Superporous PEI Cryogel

The epoxy–amine reaction is common and involves the nucleophilic addition reaction of amine to epoxy groups.[45] The oxirane rings of the epoxy component are opened by hydrogen atoms of the amine-containing component.[49] PEI cryogels were synthesized for the first time employing the known epoxy–amine reaction[42–44] under cryogenic conditions and the schematic presentation of the synthesis of PEI cryogels is shown in Figure 1. As illustrated in Figure 1, the oxirane rings of GDE can react with the amine groups of branched PEI chains under cryogenic conditions. In short, for PEI cryogel synthesis, a certain amount of branched PEI solution (1 mL, 50% in water, M_n: 1200, M_n: 1800, and M_n: 60,000) diluted to 9 mL with DI water were placed into a vial and vortexed for 2 min. Then, these solutions were cooled in a deep freezer for about 3 min and a certain amount of epoxy crosslinker, GDE 10% of mole of the repeating unit of PEI, was added and vortexed, and then these solutions were placed quickly into plastic straws of ~8 mm in diameter, and then these plastic straws were left at −18 °C for 16 h to complete cryopolymerization.

The pore structures of PEI cryogels were investigated by visualization via SEM measurements and the corresponding photographs and SEM images are shown in Figure 2(a–c) of the PEI cryogels prepared from MW, e.g., M_n: 1200, M_n: 1800, and M_n:

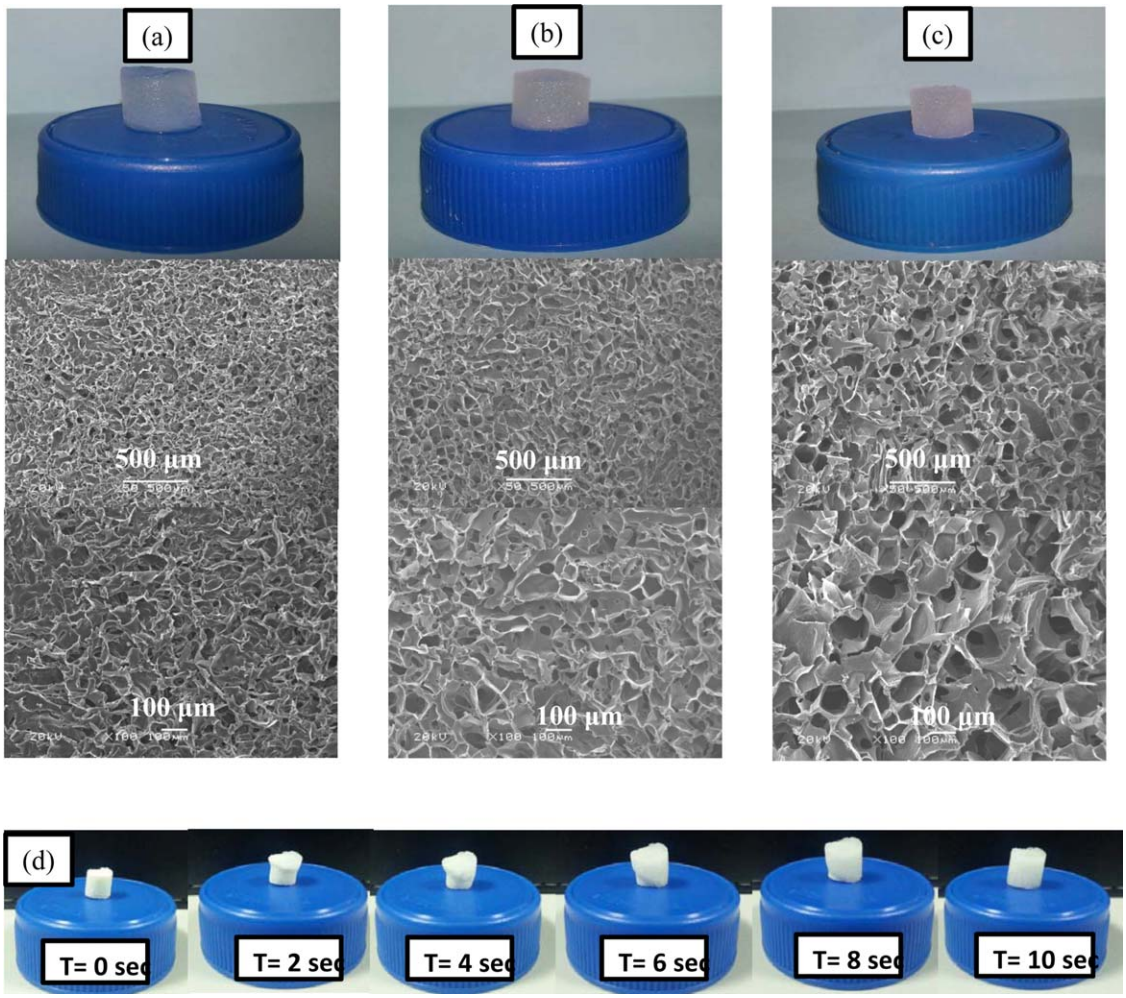

Figure 2. The photographs and SEM images of PEI cryogels synthesized from (a) M_n: 1200, (b) M_n: 1800, and (c) M_n: 60,000 branched PEI solutions, and (d) the photographs of swelling PEI cryogels synthesized from branched PEI with M_n: 1800. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

60,000 PEI solutions, respectively. It is obvious from the SEM images that, regardless of the MW of the branched PEI used, PEI cryogels have pore sizes ranging from 40 to 200 μm.

It is obvious that the PEI cryogels have superporous interconnected pore sizes up to a few hundreds of microns.[50] Super porosity in materials provides many advantages such as fast response to exterior stimuli, i.e., solvent, pH, temperature or solute molecules, and may provide high elasticity and high mechanical strength etc., in comparison to common hydrogels.[39] The DI water swelling of PEI cryogel images were acquired by dropping DI water droplets onto cryogels. As illustrated in Figure 2(d) via the camera images of swelling behavior of PEI cryogels synthesized from PEI with M_n: 1800, it took 10 s reach S_{max} % in DI water. Therefore, due to the super porosity, interconnected pore sizes and hydrophilicity of the functional groups, cryogel swelling is much faster than bulk hydrogel where sometimes bulk hydrogel swelling continues for hours and even days. Here, the prepared PEI cryogels completed their maximum % swelling in about 10 s and maxima were 1889 ± 334, and 1724 ± 144 for M_n: 1800, and M_n: 60,000 PEI solutions, respectively. The swelling %, densities, pore volume %, and porosity % of PEI cryogels synthesized from M_n: 1200 PEI solution were not calculated due to collapse of pores of the PEI cryogels during the drying process. On the other hand, for PEI cryogels that were prepared from branched PEI with Mw of M_n: 1800 and M_n: 60,000, the densities (dry and swollen), pore volume %, and porosity % values are given in Table I.

The densities of dry and swollen PEI cryogels were calculated by using eq. (2) and it was found that these values were 0.23 ± 0.05, 0.15 ± 0.01 for dried and 1.17 ± 0.1, 1.09 ± 0.1 g/cm^3 for swollen PEI cryogels prepared from branched PEI with MW of M_n: 1800 and M_n: 60,000, respectively. The pore volume % of PEI cryogels were determined by using eq. (3) and were 79.2 ± 2.7 and 71.9 ± 2.3% for PEI cryogels prepared from branched PEI with MW of M_n: 1800 and M_n: 60,000 PEI, respectively. Cyclohexane was used as a solvent in the determination of pore volume % value. As cyclohexane is a poor solvent for PEI, PEI polymer chains do not swell in cyclohexane and only superpores are filled with cyclohexane. Therefore, the amount of cyclohexane in PEI cryogel is assumed to be the

Table I. The Swelling % Ability, Density (Dry and Swollen), Pore Volume %, and Porosity % Values of PEI Cryogel

Material (PEI cryogels)	Swelling (%)	Density (g/cm^3) (dry)	Density (g/cm^3) (swollen)	Pore volume (%)	Porosity (%)	Durability
M_n: 1200	–					Not good
M_n: 1800	1889 ± 334	0.23 ± 0.05	1.17 ± 0.1	79.2 ± 2.7	67.4 ± 5.2	Very good
M_n: 60,000	1724 ± 144	0.15 ± 0.01	1.09 ± 0.1	71.9 ± 2.3	80.9 ± 0.9	Fragile

pore volume of the PEI cryogels.[51] To determine the % porosity, eq. (4) was used, and it was calculated as 67.4 ± 5.2 and 80.9 ± 0.9% for the PEI cryogels prepared from branched PEI with Mw of M_n: 1800 and M_n: 60,000, respectively. It is obvious that the MW of PEI has a significant effect on % porosity of the cryogel; the higher the MW of PEI, the higher % pore volume is obtained. The weight of cryogel after squeezing ($M_{squeezed}$) to free water from the swollen cryogel was used to estimate the total volume of macropores in the swollen cryogels. In spite of high porosity %, and pore volume % values, the durability of PEI cryogels that are synthesized with M_n: 60,000 is not good, and they are very fragile in comparison to PEI prepared from branched PEI of MW of M_n: 1800. The PEI with M_n: 60,000 in solution approximately contain 1/33 fold less PEI molecules then those prepared from M_n: 1800 PEI in solution. Therefore, the higher number of molecules in the case of M_n: 1800 PEI solution provides more chain ends and better crosslinking capability with GDE crosslinker. As the collapse of pores of PEI cryogels prepared from branched PEI with M_n: 1200 supports this idea as more crosslinking with GDE is possible with lower MW PEI, the structure becomes more brittle. Therefore, for PEI cryogel preparation, the MW of branched PEI with M_n: 1800 seems to be the critical MW and above this value MW up to M_n: 60,000 provides better PEI cryogel network. The mechanical strength of PEI cryogels synthesized from M_n: 1800 is better than M_n: 1200 and M_n: 60,000. Therefore, throughout this investigation, PEI cryogels synthesized from M_n: 1800 were used for further investigations.

To confirm the cryogel of PEI is formed, the FTIR spectrums of branched PEI and PEI-based cryogels were compared and the results are illustrated in Figure 3(a). The branched PEI chains contain primary, secondary, and tertiary amine functional groups. Some of these secondary and primary amine groups were used in crosslinking with epoxy groups of the crosslinker, GDE. It is very well known and also shown in the literature that primary, secondary, and tertiary amines can react with epoxy groups, and secondary alcohols are shown on the product.[43,44] From the FTIR spectra, the most important peaks belonging to branched PEI are C-NH_2 stretching peak at 1043 cm^{-1}, C-N-C stretching peak at 1117 cm^{-1}, N-H stretching at 3291 cm^{-1}, and C-H stretching at 2942 and 2833 cm^{-1} are clearly seen. On the other hand, upon crosslinking PEI with GDE, the most distinct peaks are 1051 and 1109 cm^{-1} coming from alcohol groups, for O-H bending and C-O stretching from secondary alcohol, respectively. PEI cryogels were also treated with 200 mL of 1.0*M* NaOH, and 0.1*M* HCl for 4 h assess the generated peaks as given in Figures 3(a) and [4], respectively, and the corresponding peaks are shown in the figure. The quarternized amine peaks are clearly seen at 1660 cm^{-1} for all the PEI materials.

Additionally, the thermal behavior of PEI-based cryogels were investigated by using TGA, heating the samples up to 750 °C at 10 °C/min heating rate under N_2 flow of 200 mL/min, and the corresponding graph is given in Figure 3(b). As PEI-based materials are highly hydroscopic, TGA analysis started at 90 °C. It is apparent from TGA curves that the degradation of bare PEI-cryogel started from 100 to 209 °C with about 13% weight loss, and the degradation continued linearly up to 650 °C with about 99% weight loss. The PEI cryogel treated with HCl and NaOH had lesser amounts of weight loss % compared to bare PEI cryogel. HCl-treated PEI cryogel started to degrade from 146 to 250 °C with 11.5% weight loss, and the greatest degradation started from 270 to 397 °C with 82.7% weight loss, and the last step of degradation of HCl-treated PEI cryogel started at 415 °C and up to 750 °C the weight loss was 95.9%. For the NaOH-treated PEI cryogels, the first degradation interval started between 188 and 206 °C with 11.9% weight loss and continued to degrade with sharp degradations between 314 and 346 °C and between 383 and 402 °C with 49.1 and 67.2% weight losses, respectively, observed. At the end of the degradation steps, NaOH-treated PEI cryogels had 83.3% weight loss at 750 °C. Therefore, the NaOH- and HCl-treated PEI cryogels have more degradation steps compared to bare PEI cryogels and thermal stabilities were also better than bare PEI cryogels. The corresponding degradation temperature ranges and weight loss % values are summarized in Table II.

The Preparation and Characterization of Ionic Liquid PEI Cryogels

In general, poly ionic liquids (PILs) can be prepared in two different ways. The first one is polymerization of ionic liquid monomers, and the second one is post modification (e.g., anion exchange reaction) of polymers.[35] In this study, the PEI-based PILs were prepared by anion exchange of the protonated anions (Cl^-) of the PEI cryogels by using IL-forming compounds such as AHFP, KSCN, SDCA, and STFB as the sources of the anions hexafluorophosphate $[PF_6]^-$, thiocyanate $[SCN]^-$, dicyanamide $[^-N(CN)_2]$, and tetrafluoroborate $[BF_4]^-$, respectively. Briefly, a certain amount of PEI cryogels, 1 g (prepared from MW of M_n: 1800 branched PEI) were treated with 200 mL 1*M* HCl solution for 4 h at 300 rpm stirring for protonation of amine groups on the PEI cryogel network. After washing protonated PEI cryogels with DI water, the anion solutions of IL source-forming compounds with 1 : 1.5 mole ratio of PEI repeating units were prepared in 100 mL water, and used for anion exchange reactions. The protonated PEI cryogels were placed in the anion solutions in water for 24 h at 300 rpm stirring, and the anion-exchanged

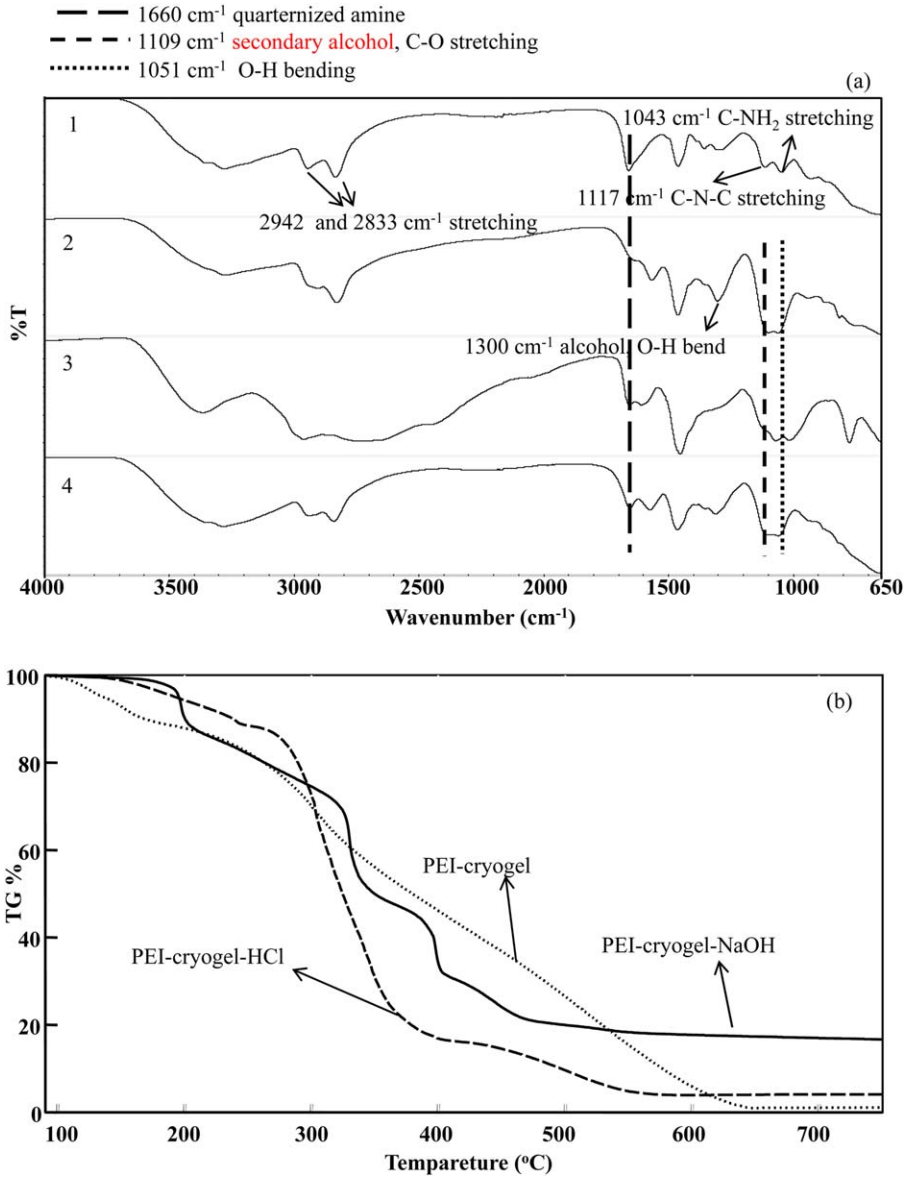

Figure 3. (a) The FTIR spectra of PEI-based cryogels (1-branched PEI, 2-PEI-cryogel, 3-PEI-cryogel-NaOH, and 4-PEI-cryogel-HCl) and (b) TGA thermograms of PEI-based cryogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cryogels were washed with excess DI water three times for 1 h under 300 rpm mixing rate, and dried in an oven at 50 °C for further use. The schematic presentation of the preparation of PILs from PEI cryogels is shown in Figure 4. Each of the protonated amine groups (cation) contains a negative Cl^- ion as shown in the protonation step in Figure 4(a). After protonation, the anion exchange reaction with the anions of $[PF_6]^-$, $[^-SCN]$, $[^-N(CN)_2]$, and $(BF_4)^-$ replaced Cl^- anions within the PEI cryogel network, and the corresponding reaction schemes for each anion are given in Figure 4(b–e), respectively.

As shown in Table III, swelling %, density (dry and swollen), pore volume %, and porosity % values of HCl-treated PEI, NaOH-treated

Table II. Thermal Degradation Temperature Ranges and % Weight Losses of PEI and PEI Treated with HCl and NaOH Cryogels

	Thermal degradation temperature ranges (°C) and % weight losses			
Cryogels	I.	II.	III.	IV.
PEI-bare	100-209 13	213-649 99	653-750 99.2	-
PEI-HCl	146-250 11.5	270-397 82.7	415-562 95.6	580-750 95.9
PEI-NaOH	188-206 11.9	314-346 49.1	383-402 67.2	465-750 83.3

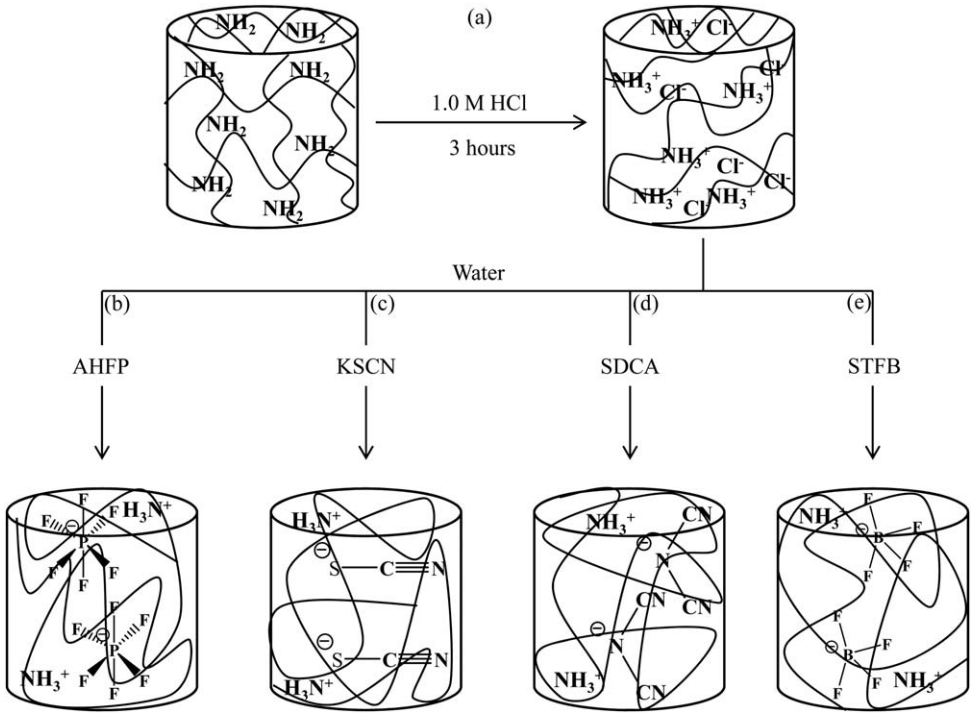

Figure 4. The schematic presentation of (a) protonated superporous PEI cryogels with HCl treatment, and the superporous PEI cryogels anion exchanged with (b) AHFP, (c) KSCN, (d) SDCA, and (e) STFB.

PEI, and modified PEI (anion exchanged cryogels) were compared with each other. HCl- and NaOH-treated PEI cryogels have swelling % values of 1124 ± 243 and 1393 ± 49.8, respectively, and these values are decreased significantly after anion exchange. The swelling % values of anion exchanged PEI cryogel decreased from 1124 ± 243 to 521 ± 21.1%, 614 ± 13.8, 152 ± 40.5, and 772 ± 17.3% for Cl^- to $[PF_6]^-$, $[SCN]^-$, $[N(CN)_2]^-$, and $[BF_4]^-$, respectively. In addition, the pore volume % values also decreased and the highest decrease for DCA anions could be due to the hydrophobicity of this anion in comparison to the other anions. The same effect is also observed for porosity % with the same anion, whereas the other anions did not show significant reduction in porosity % values as obvious from the detailed values given in Table III.

To confirm the anion exchange reactions, FTIR spectra of modified PEI cryogels were taken and compared with the protonated PEI cryogels and the corresponding spectra are illustrated in Figure 5. It is clear from Figure 5(a) that the anion-exchanged PEI cryogels possess specific peaks for their corresponding anion functional groups. As can be seen, the $[PF_6]^-$ anion-containing protonated PEI cryogel has one distinct peak at 839 cm^{-1} belonging to P-F stretching, and the $[SCN]^-$ stretching peak at 2055 cm^{-1} for thiocyanate for the matching modified PEI cryogels. Also, different from the others, the three important peaks for nitrile stretchings, unconjugated nitrile and conjugated nitrile peaks at 2139, 2201, and 2240 cm^{-1}, respectively, belonging to $[N(CN)_2]^-$ for SDC anion-exchanged PEI cryogels are clearly seen. The B-F peak for $[BF_4]^-$ anions at 1052 cm^{-1} is also obviously seen. Therefore, PILs of PEI cryogels are confirmed by the peaks on the FTIR spectra which are in accordance with the literature.[5–9]

Furthermore, the TGA thermograms for the modified PEI cryogels were also taken to investigate the effects of different anions such as Cl^-, $[PF_6]^-$, $[SCN]^-$, $[N(CN)_2]^-$, and $[BF_4]^-$ on modified PEI cryogels and these are illustrated in Figure 5(b). It is evident that the PEI cryogels with $[PF_6]^-$ and $[BF_4]^-$ anions have higher thermal stability with lesser weight losses of about 85.8%

Table III. The Swelling % Ability, Density (Dry and Swollen), Pore Volume %, and Porosity % Values of PEI-Based Poly(IL) Cryogels

Material	Swelling (%)	Density (g/cm^3) (dry)	Density (g/cm^3) (swollen)	Pore volume (%)	Porosity (%)
PEI-HCl	1124 ± 243	0.33 ± 0.1	1.35 ± 0.19	91.6 ± 1.5	65.9 ± 6.5
PEI-NaOH	1393 ± 49.8	0.21 ± 0.03	0.85 ± 0.18	93.3 ± 0.18	73.81 ± 2.7
PEI^+ -$[PF6]^-$	521 ± 26.1	0.43 ± 0.01	0.92 ± 0.06	83.8 ± 0.7	67.3 ± 1
PEI^+ -$[^-SCN]$	614 ± 13.8	0.35 ± 0.02	0.87 ± 0.05	85.9 ± 0.3	70.9 ± 2.8
PEI^+ -$[^-C(CN)_2]$	152 ± 40.5	0.38 ± 0.05	0.96 ± 0.1	59.6 ± 5.9	22.2 ± 12.5
PEI^+ -$[BF_4]^-$	772 ± 17.3	0.35 ± 0.02	1.06 ± 0.05	88.5 ± 0.2	65.7 ± 1.9

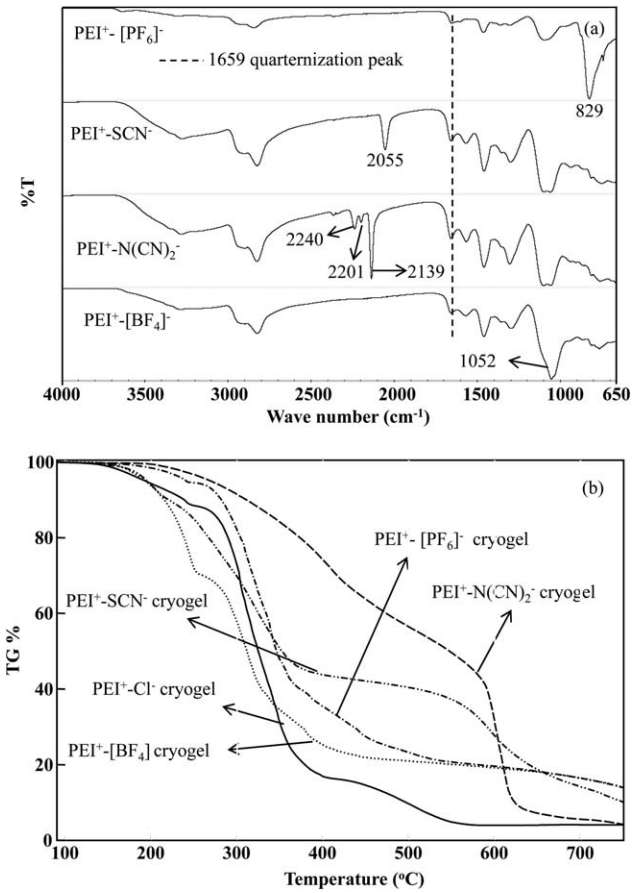

Figure 5. (a) The FTIR spectra of PEI-based anion exchanged cryogels: PEI^+-$[PF_6]^-$ cryogel, PEI^+-$[SCN]^-$ cryogel, PEI+[-$N(CN)_2$] cryogel, and PEI^+-$[BF_4]^-$ cryogel, and (b) TGA thermograms of PEI-based anion exchanged cryogels.

in comparison to the other anion-containing modified PEI cryogels such as Cl^-, $[SCN]^-$, $[N(CN)_2]^-$ with 95.9, 89.8, and 95.8% weight losses at 750 °C. The details of degradation temperature ranges with % weight lose are given in Table IV. As can be seen, the types of anions in the modified PEI cryogels significantly affect thermal stability of PILs.

Bovine Serum Albumin Absorption

BSA is a serum albumin protein obtained from cows. It is often used as a protein concentration standard in laboratory experiments, including many biochemical applications such as ELISA (enzyme-linked immunosorbent assay), immune blots, immunohistochemistry, and nutrients in cell and microbial cultures.[52] Therefore, the absorption of BSA with prepared PEI cryogels was investigated. The pH of prepared BSA solution in water was measured as 7.3 by using pH meter (Thermo, ORION 5 Star). For the batch-type absorption of BSA, a certain amount of PEI cryogels, 0.05 g, is placed into 20 mL 300 ppm BSA solution under 100 rpm mixing rate for 4 h. The amounts of absorbed BSA were calculated by using a UV–vis spectrophotometer via previously constructed calibration curve at 280 nm and corresponding UV–vis spectra of BSA solution in DI water is given in Supporting Information Figure 1(a). Furthermore, the BSA adsorption from DI water by bare and anion exchanged PEI based microgels is given in Supporting Information Figure 1(b), and the maximum adsorbed amounts of BSA are given in Table V. As can be seen from Table V, 36.4 ± 1.7 mg/g BSA per gram superporous bare PEI cryogel is absorbed and this amount is better than in the literature.[53] Of the modified PEI cryogels on the other hand, the PEI cryogels modified with Cl^- and $[PF_6]^-$ also absorbed slightly higher amounts of BSA under the same conditions; 39.9 ± 3.6, and 47.8 ± 5.7 mg/g as given in Table V. In general, proteins tend to adsorb more strongly to non-polar surfaces than polar ones with high surface tension than to low surface tension, as well as more to the charged substrates then to uncharged ones.[54] As, the modification of PEI cryogels were done for the preparation of PEI based ionic liquids, and the ionic liquids are generally described as hydrophobic in comparison to the original cationic or particles anion unchanged chloride salt forms. By modification, e.g., treatments with HCl, most of amine groups are charged and then neutralized with small charged Cl^- ions, and with anion exchange reactions, the more hydrophobic and bulkier anions such as $[PF_6]^-$, $[SCN]^-$, $[N(CN)_2]^-$, and $[BF_4]^-$ are replaced with Cl^- anions, hence, the modified PEI ionic liquids become more hydrophobic.[55] Therefore, we suggested the hydrophobicity of PEI cryogels increased due to electrostatic interactions between the positively charged quaternary amine groups and Cl^-, $[PF_6]^-$, $[SCN]^-$, $[N(CN)_2]^-$, and $[BF_4]^-$ anions resulted in higher amounts of BSA adsorption in comparison to unmodified PEI cryogels. The other anion-containing modified cryogels such as $[SCN]^-$ and $[N(CN)_2]^-$ did not absorb any BSA at all. The least amount of BSA was absorbed by $[BF_4]^-$ with 9.2 ± 1.5 mg/g absorbed under the same conditions. Because of the nature of these anions and their interactions with BSA, there is huge difference in absorption capacities of BSA for the different anion-containing superporous PEI cryogels.

Table IV. Thermal Degradation Ranges of the Modified PEI Cryogels with % Weight Losses

Materials [poly(IL) cryogels]	Thermal degradation temperature ranges (°C) and weight losses (%)			
	I.	II.	III.	IV.
PEI^+ -Cl^-	146- 250 11.5	270- 397 82.7	415- 562 95.6	580- 750 95.9
PEI^+ -$[PF_6]^-$	154- 252 29.1	270- 407 76.4	410- 750 85.8	-
PEI^+ -[$^-$SCN]	143- 374 54.5	555- 656 81.9	660- 750 89.8	-
PEI^+ -$N(CN)_2^-$	196- 562 52.4	576- 624 90.3	631- 750 95.8	-
PEI^+ -$[BF_4]^-$	167- 241 5.1	258- 374 60.1	398- 750 85.8	-

Table V. The Effect of Anion Exchange on Absorption of BSA by PEI-Based Cryogels

	PEI cryogels with different anions					
Molecules	Bare	$[Cl]^-$	$[PF_6]^-$	$[^-SCN]$	$[^-N(CN)_2]$	$[BF_4]^-$
Bovine serum albumin (mg/g)	36.4 ± 1.7	39.9 ± 3.6	47.8 ± 5.7	-	-	6.7 ± 0.6

The Removal of Organic Dyes from Aqueous Media by PEI Cryogels

The removal and separation of organic dyes from aquatic environments is important due to their adverse effect on humans and other living organisms, and they are known to have toxic and carcinogenic effects.[47,48] Therefore, the removal of organic dyes such as EY, MO and MB from aqueous media was investigated, and corresponding UV–vis absorption spectra together with their chemical formulae are illustrated in Figure 6(a–c), respectively. EY, MO and MB have maximum absorption wavelengths at 514, 464, and 664 nm. It is clear that positively charged PEI cryogel can inherently absorb negatively charged species such as EY and MO organic dyes. The EY solutions of $16 \times 10^{-5}M$, MO $40 \times 10^{-5}M$, and MB $5.2 \times 10^{-5}M$ with 15 mL volume were prepared

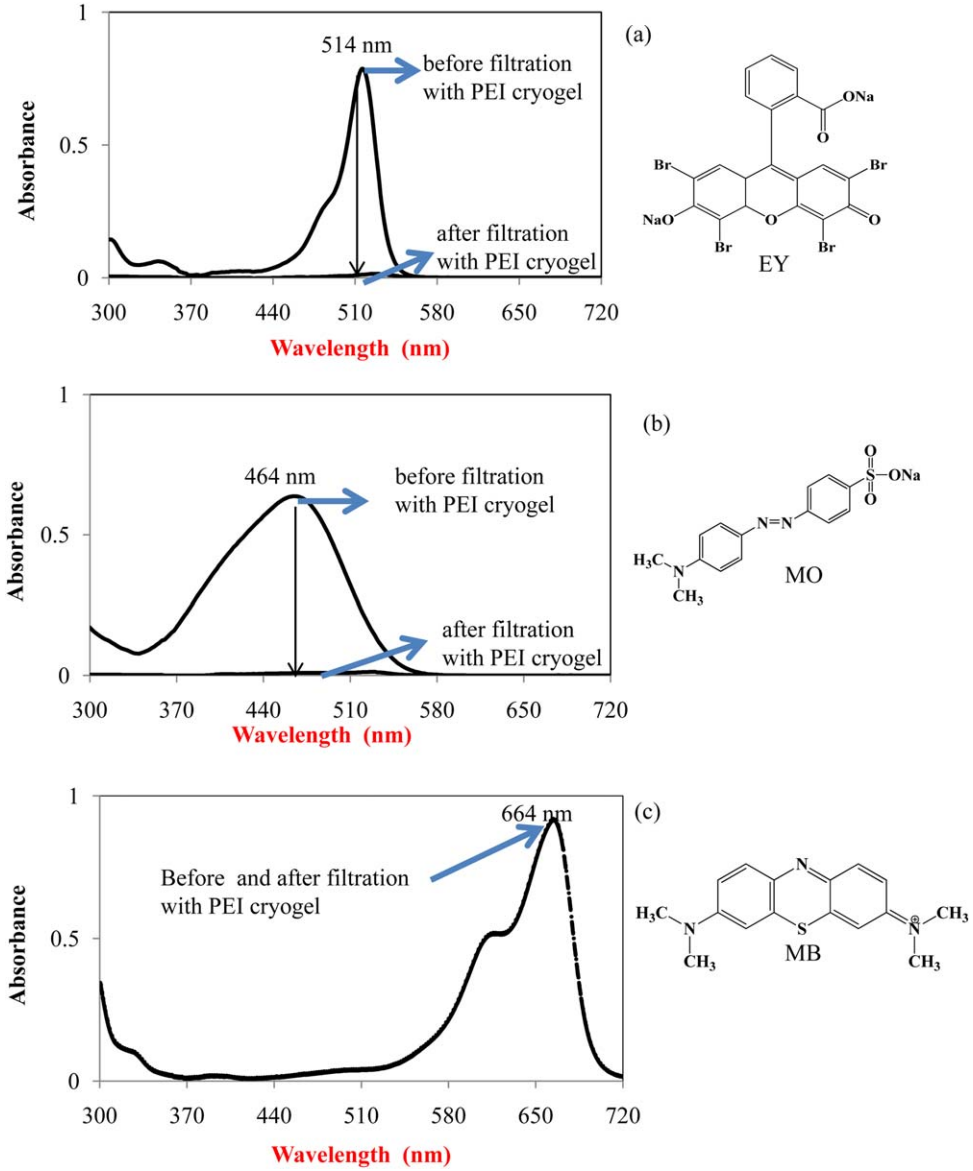

Figure 6. The UV–vis spectra and chemical structure of (a) eosin Y (EY), (b) methyl orange (MO), and methylene blue (MB) before and after passing through pasteur pipettes each containing 0.025 g PEI cryogels (15 mL of $16 \times 10^{-5}M$ EY, $40 \times 10^{-5}M$ MO, and $5.2 \times 10^{-5}M$ of MB in DI water). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

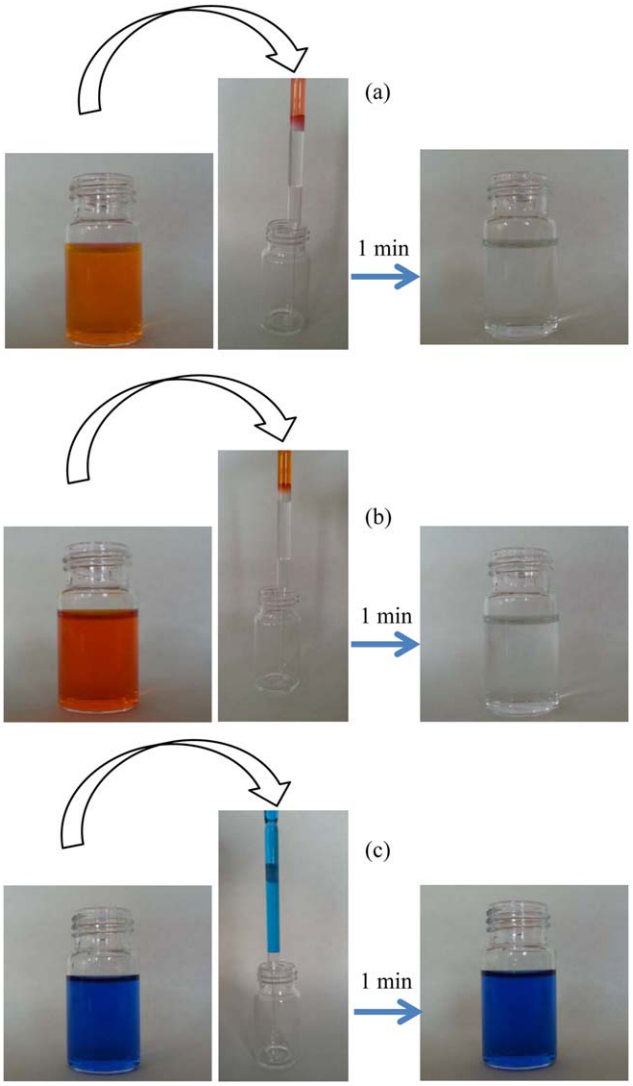

Figure 7. The photographs of purification of organic dyes such as (a) EY, (b) MO, and (c) MB from their corresponding aqueous solutions (15 mL of $16 \times 10^{-5}M$ EY, $40 \times 10^{-5}M$ MO, and $5.2 \times 10^{-5}M$ of MB in DI water). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in DI water and their solutions were passed through pasteur pipettes filled with 0.025 g of dried PEI cryogels under the effect of gravity. As illustrated in Figure 6 (a,b), the dashed lines show no corresponding peaks upon simple filtration of EY and MO dyes via PEI cryogel-containing column (PEI filled pasteur pipettes filled). To pass 15 mL of these dye solutions through 0.025 g PEI cryogel-filled pipettes takes about 1 min. It was calculated that removal efficiencies of 98.5, and 98.6% were obtained for EY, and MO, respectively. As illustrated in Figure 6(c), 15 mL MB at $5.2 \times 10^{-5}M$ concentration passed through the same column e.g., PEI-filled pasteur pipettes, and there was no dye absorption and no reduction at the corresponding maximum absorption wavelength of 664 nm as shown in the figure. Therefore, it can be presumed that PEI cryogels possess great potential as purification and separation materials.

To visualize EY and MO dye removal by simple filtration systems using superporous PEI cryogel system, photographs of the aforementioned systems [Fig. 6(a–c)] are illustrated in the same order in Figure 7(a–c), respectively.

It is clear from Figure 7(a,b) that after passing EY and MO dye solutions through PEI cryogel-containing columns under the force of gravity, the dyes are completely retained by PEI cryogels within min (~1 min) losing their color and leaving clear water as shown on right hand side for (a) and (b) of the figures. On the other hand, there is no change in color of the MB solution after passing through the same column containing PEI cryogels is shown in Figure 7(c). So, it is obvious that the interaction between PEI cryogels and dye molecules is electrostatic in nature.

Separation of MB from MO-MB Mixture and MO-MB-EY Mixtures

To further demonstrate that superporous cryogels can be used in separation of toxic chemicals, such as organic dyes, because of their easy, simple, and practical usability as column filler materials,[56,57] the separation of MB from mixtures of dye solutions was investigated. For this purpose, 7.5 mL of MB and MO solutions at $5.2 \times 10^{-5}M$ and $40 \times 10^{-5}M$ concentration were mixed and passed through a column as illustrated in Figure 8(a) with their corresponding photographs. Upon mixing MB (blue colored) and MO (orange), a dark brown color is generated as can be seen in the corresponding photographs in Figure 8(a), and after passing through a column under the force of gravity, pure MB is obtained. This is confirmed with UV–vis spectroscopy. It is obvious that the UV–vis absorption peak at 466 nm for MO disappears after filtration (the dashed line in the figure) and the peak at 664 nm is retained belonging to MB. The absorbance value of MB at 664 nm was 0.218, after the separation process, the maximum absorbance values for MB at 664 nm was 0.250. The slight increase in the absorption of the peak for MB could be due to the interaction of both dyes with each other as they are oppositely charged.

Moreover, the separation of MB from a triple dye mixture was also investigated using the mixture of MO-MB-EY. For this purpose, 5 mL of each dye with $16 \times 10^{-5}M$ EY, $40 \times 10^{-5}M$ MO, and $5.2 \times 10^{-5}M$ MB concentrations were mixed, and this mixture was passed through a column containing 0.025 g PEI cryogels as shown in Figure 8(b), together with corresponding photographs and UV–vis spectra. It is remarkable that pure MB is obtained from these MO-MB-EY mixtures by a simple filtration system. From the UV–vis spectrum of the mixture of three dyes, MO and MB slightly red shifted (higher wave lengths), e.g., MO from 464 to 484 nm and MB from 664 to 670 nm in the mixture. Upon filtration of this dye mixture, 98 and 97% of MO and EY were removed, respectively, by this simple separation system. The MB in the mixture shifted so that maximum absorbance wave length was 670 nm and after filtration this shift disappeared and MB was absorbed at the same wave length of 664 nm confirming the electrostatic interaction of these dyes (MO-MB and EY-MB). Therefore, it is apparent

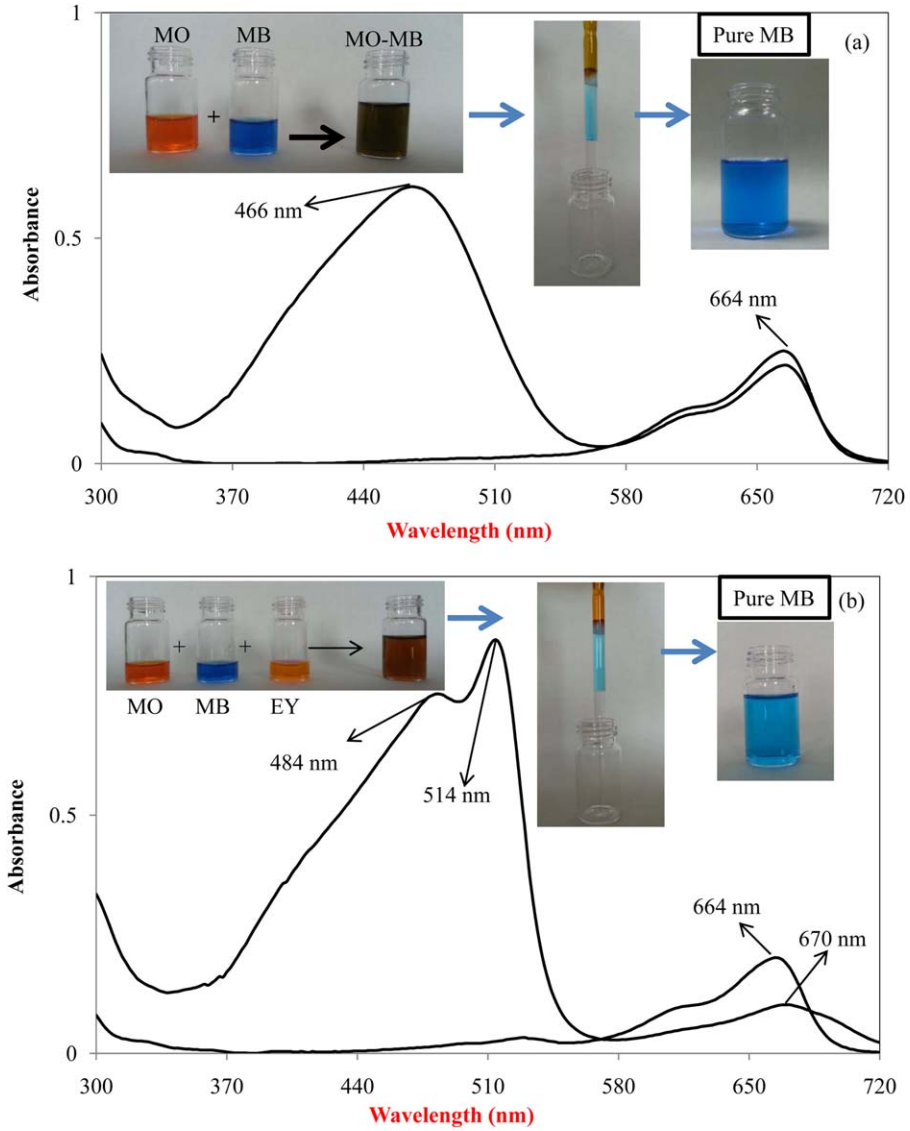

Figure 8. The UV–vis spectra and photographs of separation of MB from organic dye mixtures (a) MO-MB and (b) MO-MB-EY using PEI cryogels as column filler. [$16 \times 10^{-5}M$ EY, $40 \times 10^{-5}M$ MO, and $5.2 \times 10^{-5}M$ MB in (a) 7.5 mL of MO and MB, and in (b) 5 mL of MO, MB, and EY]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that PEI-based superporous cryogels are indispensable materials for the fast separation of organic dyes.

CONCLUSIONS

In this study, we reported the facile synthesis of superporous PEI cryogels with an epoxy–amine reaction under cryogenic conditions for the first time. Three different molecular weight (M_n: 1200, M_n: 1800, and M_n: 60,000) PEI solutions were used for PEI cryogel preparation and were compared for their maximum swelling %, densities (dry and swollen condition), pore volume %, porosity % values, and mechanical strength. It was found the critical molecular weight of branched PEI between M_n: 1200 and M_n: 60,000 provided optimum PEI cryogels with better physical properties, e.g., $1889 \pm 334\%$ swelling, $79.2 \pm 2.7\%$ pore volume, $67.4 \pm 5.2\%$ porosity with better mechanical strength. Furthermore, PILs were prepared from protonated PEI cryogels by anion exchange of protonated PEI with hexafluorophosphate ($[PF_6]^-$), thiocyanate $[SCN]^-$, dicyanamide $[N(CN)_2]^-$, and tetrafluoroborate ($[BF_4]^-$) anions in aqueous environments. It was also demonstrated here that PEI cryogels have increased absorption capacity for BSA from 36.4 ± 1.7 to 39.9 ± 3.6 and 47.8 ± 5.7 mg BSA per gram for bare PEI cryogels exchanged with $[Cl^-]$ and $[PF_6]^-$ from aqueous solutions.

Furthermore, it was also demonstrated here that superporous PEI cryogels are very resourceful for the removal of organic toxic dyes such as EY and MO organic dyes from their aqueous solutions with 98.5 and 98.6% removal efficiency from 15 mL of $6 \times 10^{-5}M$ EY, $40 \times 10^{-5}M$ MO dye solutions within minutes. More interestingly, it was also proven here that the superporous PEI cryogels can be used as column filler materials

for the separation of organic dyes, e.g., the separation of MB from MO-MB binary and MO-EY-MB tertiary mixtures within one minute. Therefore, it was demonstrated that superporous PEI cryogels have great potential in separation and purification applications and for elimination or removal of certain species in environmental and chromatography applications, as well as rapid detection and elimination of drugs, biological molecules, hormones, proteins, pesticides, and so on.

ACKNOWLEDGMENTS

This project is supported by The Scientific and Technological Research Council of Turkey (TUBITAK-214M130).

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