Synthesis and Characterization of Poly(N-isopropylacrylamide) Thermosensitive Based Cryogel

Poli(N-izopropilakrilamid) bazlı kriyojelin sentez ve karakterizasyonu

Research Article

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ABSTRACT

The aim of this study was to reveal the changes in the nanostructure of the thermo-sensitive poly(N-isopropylacrylamide-N methacryloyl-(L)-histidine) [poly(NIPA-MAH)] monolithic cryogel with changing temperature around lower critical solution temperature (LCST) of NIPA. Poly(NIPA-MAH) cryogel was prepared by free radical cryopolymerization of NIPA with MAH as a functional comonomer and N,N-methylene-bisacrylamide (MBAAm) as crosslinker directly in a plastic syringe. Cryo-polymerization was initiated by N,N,N,N-tetramethylene diamine (TEMED) and ammonium persulfate (APS) pair at subzero temperature (-16°C) in an ice bath. LCST of poly(NIPA-MAH) cryogel was found to be 34°C. The surface morphology and bulk structure of poly(NIPA-MAH) cryogel was revealed with scanning electron microscopy (SEM). Poly(NIPA-MAH) cryogel with 60–100 μm pore diameter has a specific surface area of 42.6 m^2/g polymer. Poly(NIPA-MAH) cryogel was characterized by Fourier transform infrared spectrometer (FTIR) and swelling test. The equilibrium swelling degree of poly(NIPA-MAH) cryogel was 22.08 g H_2O/g for dry cryogel. The presence of NIPA makes cryogel morpholgy highly porous and differentiates it from conventional gels.

Key Words

N-isopropylacrylamide (NIPA), thermosensitive polymers, cryogels, histidine.

ÖZET

Bu çalışmanın amacı NIPA'nın düşük kritik çözelti sıcaklığı (LCST) civarındaki sıcaklık değişimlerine karşı pH'ya ve sıcaklığa duyarlı poli(N-izopropilakrilamid-N metakriloil-(L)-histidin) [poli(NIPA-MAH)] monolitik kriyojelin nanoyapısındaki değişimleri açıklamaktır. Poli(NIPA-MAH) kriyojel plastik şırınga içerisinde NIPA'nın fonksiyonel komonomer olarak kullanılan MAH ile serbest radikal kriyokopolimerizasyonu ile hazırlanmış olup N,N-metilen-bisakrilamid (MBAAm) çapraz bağlayıcı olarak kullanıldı. Kriyopolimerizasyon N,N,N,N-tetrametilen diamin (TEMED) ve amonyum persülfat (APS) çifti ile buz banyosunda sıfırın altındaki sıcaklıkta (-16°C) başlatıldı. Poli(NIPA-MAH) kriyojelin LCST değeri 34°C olarak bulundu. Poli(NIPA-MAH) kriyojelin yüzey morfolojisi ve yığın yapısı taramalı elektron mikroskobu (SEM) ile belirlenmiştir. 60–100 μm por çapına sahip poli(NIPA-MAH) kriyojel 42.6 m^2/g polimer değerinde yüzey alanına sahiptir. Poli(NIPA-MAH) kriyojel Fourier dönüşümlü kızılötesi spektrometre (FTIR) ve şişme testi ile karakterize edildi. Kuru kriyojelin denge şişme derecesi 22.08 g H_2O/g olarak bulundu. Geleneksel jellerle karşılaştırıldığında, bu gözenekli kriyojel şekli NIPA'ya hızlı yanıt verme olanağı sağlar.

Anahtar Kelimeler:

N-izopropilakrilamid (NIPA), sıcaklığa duyarlı polimerler, kriyojeller, histidin.

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INTRODUCTION

Intelligent thermo-sensitive and pH-sensitive gels are able to change their volume and surface structures upon slight variations in external stimuli such as temperature, pH, ionic strength, etc. [1-3]. Thermo-sensitive gels have drawn attention and found applications in the fields of drug delivery, enzyme immobilization, actuators, biomolecular separation and removal of toxic metal ions [4-8]. For instance; poly N-isopropylacrylamide (NIPA) is one of the most studied thermo-sensitive polymer with a lower critical solution temperature (LCST) of 32ºC. NIPA is hydrophilic in its water-swollen form below 32ºC while it becomes quite hydrophobic by shrinking tightly in presence of water as the temperature rises above 32ºC [9]. As the LCST of NIPA is within the range of the physiological temperature (37ºC), it has been widely used in the preparation of controlled drug delivery systems, biomaterials, and in the purification of biomolecules [10-14]. LCST of NIPA thermopolymer varies and it greatly depends on the content of the hydrophobic or hydrophilic monomers used in the polymerization step.

pH-responsive polymers have ionizable groups such as carboxylic acids or amines that can accept or donate protons in response to variations in environmental pH, ultimately changing their hydrophilic or hydrophobic natures by swelling or shrinking the polymers in presence of water at certain pHs [15-18]. For instance; histidine contains an ionizable imidazole group with a pKa value of 6.0, below which point a proton is gained while the proton is released above the pKa point, making it a suitable monomer for the production of pH-responsive hydrophilic polymers [19].

Preparation of intelligent gels is pretty much the same as that of polymeric cryogels, which are megaporous gels synthesized at temperatures below the freezing point of the solvent used [20]. In general, the polymeric cryogels have a sponge-like morphology with inter-connected many large pores being of 10-200 μm in size, which allow non-hindered diffusions of solutes, including macromolecules, as well as convective mass transport of viscous media [21-26]. Megaporous cryogels can be used in the purification of certain biomolecules of interest, including recombinant proteins and enzymes in cell lysates as well as separation of certain bacterial cells or viruses in culture media [27]. As each type of megaporous cryogels possesses a unique morphology, they can be used as the matrix of chromatography columns to separate target molecules with high-affinity, high-specificity and considerably shorter swelling/shrinkage time as compared to a conventional gel of the same chemical composition [28-32].

In several biotechnological applications NIPA intelligent gels may require further modifications on their chemical compositions in order to provide the gels with additional characteristics while their thermosensitive feature is yet preserved. In this study, N methacryloyl-(L)-histidine (MAH) was used as a co-monomer in the preparation of poly(NIPA-MAH) cryogel. The polymeric cryogel is both thermo-sensitive due to its NIPA residues and pH-responsive due to its MAH rediues in the polymer network. MAH includes histidine, an amino acid residue which can be prefentially included in chromatographic packing materials due to its polar nature, cost-effectiveness, biocompatibility, resistance to harsh chemicals, and durability at high temperatures [33]. For instance, histidine has been previously used as an efficient pseudo-affinity ligand in chromatographic purificiation of immunoglobulin G (IgG) from human plasma [34-36]. The varying properties of LCST dependent NIPA and pH dependent MAH in the poly(NIPA-MAH) cryogel novelly developed in our laboratory are schematically illustrated in Figure 1, in which the cryogel's surface becomes hydrophobic above LCST and pH > 6.0 while it becomes hydrophilic below LCST and pH < 6.0.

The poly(NIPA-MAH) cryogel was characterized and discussed thoroughly. The alteration of surface properties from hydrophilic to hydrophobic of thermosensitive poly(NIPA-MAH) was investigated by conventional methods including FTIR, SEM and swelling tests.

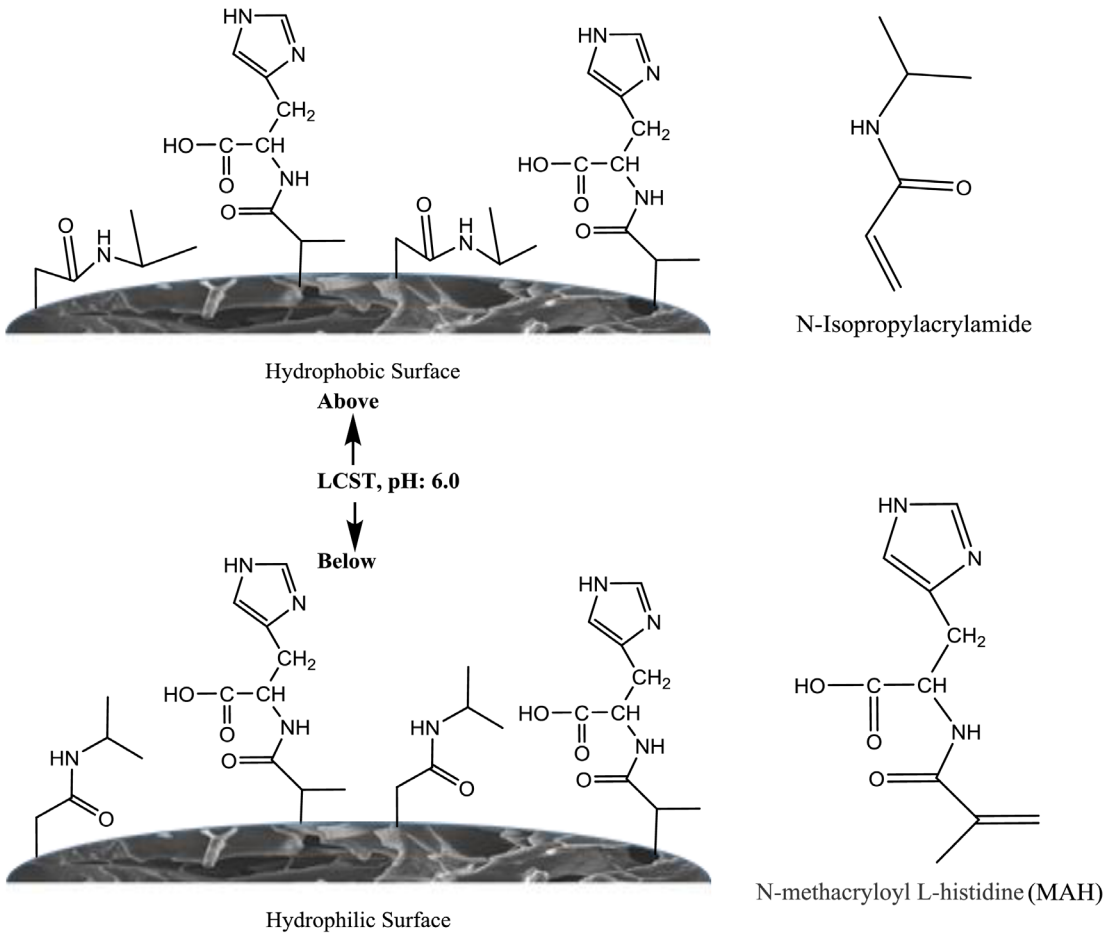

Figure 1. Schematic illustration of surface properties of the poly(NIPA-MAH) cryogel with changing temperature and pH.

EXPERIMENTAL

Materials

L-histidine, methacryloyl chloride, N,N-methylene-bis(acrylamide) (MBAAm), ammonium persulfate (APS) and wide range molecular-weight marker were supplied by Sigma Chemical Co. (St Louis, MO, USA). N-Isopropylacrylamide (NIPA), distilled under reduced pressure in the presence of hydroquinone inhibitor, was obtained from Aldrich Chem. Co. (USA), which was stored at 4ºC before use. N,N,N,N-Tetramethylene diamine (TEMED) was obtained from Fluka A.G. All other chemicals used were of reagent grade and obtained from Merck A.G. (Darmstadt, Germany) unless otherwise noted. Water used in the experiments was purified by a Barnstead (Dubuque, IA) ROpure LP reverse osmosis unit equipped with a high-flow cellulose acetate membrane (Barnstead D2731), a Barnstead D3804 NANOpure organic/colloid removal unit, and ion-exchange packed-bed system.

Synthesis of MAH monomer

Detailed information for the preparation and characterization of N methacryloyl-(L)-histidine (MAH) is reported elsewhere [33]. Briefly, the following procedure was applied for the synthesis of MAH monomer: 5.0 g of L-histidine and 0.2 g of hydroquinone were dissolved in 100 ml of CH_2Cl_2 solution, which was cooled down to 0ºC. Then, 12.74 g of triethylamine was added to the 5.0 ml of methacryloyl chloride and was slowly poured into the solution under nitrogen atmosphere, which was stirred magnetically at room temperature for 2 h. At the end, the unreacted

methacryloyl chloride was removed from the reaction mixture by 10% NaOH extraction. The aqueous phase was evaporated under vacumm by a rotary evaporator. The desired product, MAH, was crystallized in ethanol and ethyl acetate.

Preparation of poly(NIPA-MAH) cryogel

Copolymer of N-isopropylacrylamide with MAH was synthesized by free radical cryopolymerization. A typical preparation procedure is as follow: In order to prepare the monomers solution for cryogel synthesis, firstly, 300 mg of NIPA and 200 mg of MAH were dissolved in 5.0 mL of deionized water, which was mixed with a freshly prepared solution of 283 mg MBAAm in 10 mL deionized water, 20 mg of APS (1% of the total monomers, w/v), an accelerator of polymerization, was added to the solution containing the monomers, which was then cooled in an ice-bath for 5 min. The cryogel synthesis was initialized by addition of 25 μL of TEMED into the ice-cold solution and the reaction mixture was magnetically stirred for 1 min. The reaction mixture was poured into a plastic syringe with an internal diameter of 0.8 cm, a total volume of 5 mL, and a closed adaptor at the bottom, and immediately maintained at -16ºC. The polymerization was carried out for 24 h.

Characterization of the poly(NIPA-MAH) cryogels

Pore size and size distribution of the poly(NIPA-MAH) cryogel was determined by the nitrogen absorption method using the Flowsorb II instrument (Micromeritics Instrument Corporation, Norcross, USA). Specific surface area of the poly(NIPA-MAH) cryogel was determined by the multipoint Brunauer-Emmet-Teller (BET) method. A piece of the cryyogel was placed in a sample holder, oxygen was removed from the sample by passing N_2 through the sample holder for 60 min. at 150ºC. The gas absorption and desorption capacity of the cryogel was studied at -10ºC and 25ºC, respectively. Data obtained in the desorption step was used for the calculation of surface area.

Water uptake ratio of the poly(NIPA-MAH) cryogel was determined using distilled water. The swelling (water uptake) ratio of the cryogel was determined by the gravimetric method using Equation 1 in which the required parameters were determined as follows: Dried poly(NIPA-MAH) cryogel was firstly weighed out ($W_{dry\text{-}gel}$), and than immersed in distilled water at room temperature until an equilibrium was reached. The swelled cryogel was taken out of water and weighed out once again after wiping out the excess water on the cryogel surface by filter paper ($W_{wet\text{-}gel}$). The swelling ratio of poly(NIPA-MAH) cryogel was calculated using the following equation;

$$\text{Water uptake ratio \%} = [(W_{wet\text{-}gel} - W_{dry\text{-}gel})/W_{dry\text{-}gel}].100 \quad (1)$$

FT-IR spectrum of the poly(NIPA-MAH) cryogel was taken at room temperature between 4000-400 cm^{-1} by a FT-IR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan), for which the FTIR sample was prepared as follows: About 0.1 g of dry cryogel and 0.1 g of KBr (IR Grade, Merck, Germany) were thoroughly mixed, ground, and compressed into a filmy pellet.

The surface morphology of poly(NIPA-MAH) cryogel sample, lyophilized at -50ºC by a lyophilizer instrument (Lyophilizer, Christ Alpha 1-2 LD plus, Germany), was monitored by a scanning electron microscope (SEM) instrument (JEOL JSM 5600, Tokyo, Japan).

RESULTS AND DISCUSSION

The thermo-sensitive poly(NIPA-MAH) monolithic cryogel was produced by cryopolymerization of frozen monomers N-isopropylacrylamide (NIPA) and N-methacryloyl-(L)-histidine (MAH) using N, N-methylene-bis(acrylamide) (MBAAm) as the cross-linker, amonium persulfate (APS) as the initiator of the polymerization, and N,N,N,N-tetramethylene diamine (TEMED) as the accelerator of the polymerization. The poly(NIPA-MAH) monolithic cryogel was eventually molded in a plastic syringe as an opaque, spongy and elastic polymer. To the best of our knowledge, this is the first time that the synthesis and characterization of such a poly(NIPA-MAH) cryogel is ever reported here.

The LCST of the poly(NIPA-MAH) cryogel is assumed to be the same as that of NIPA, which is 32ºC. The shrinking and swelling capacities of the poly(NIPA-MAH) cryogel were pictured at 49ºC

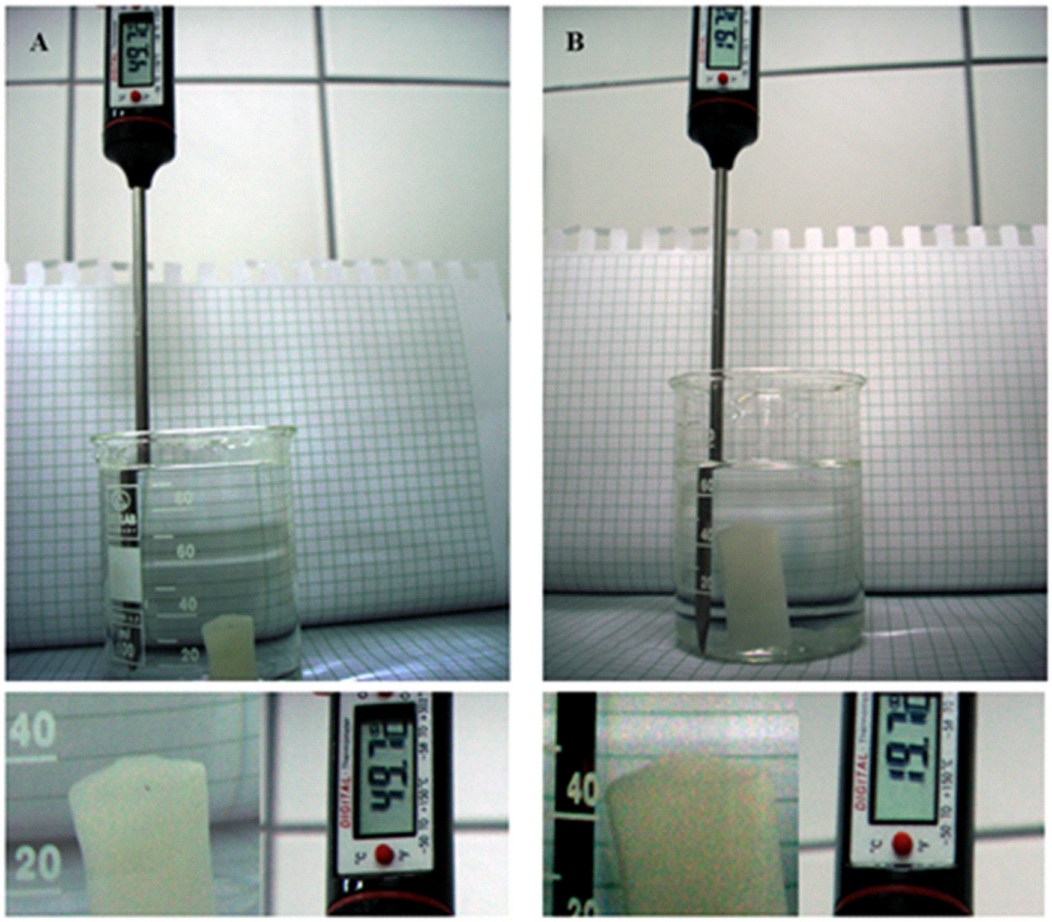

Figure 2. The shrinking and swelling properties of the poly(NIPA-MAH) cryogel placed in a beaker filled with water. The pictures were taken. A) at 49°C above LCST for monitoring the shrinked form, and B) at 19°C below LCST for monitoring the swelled form of the cryogel.

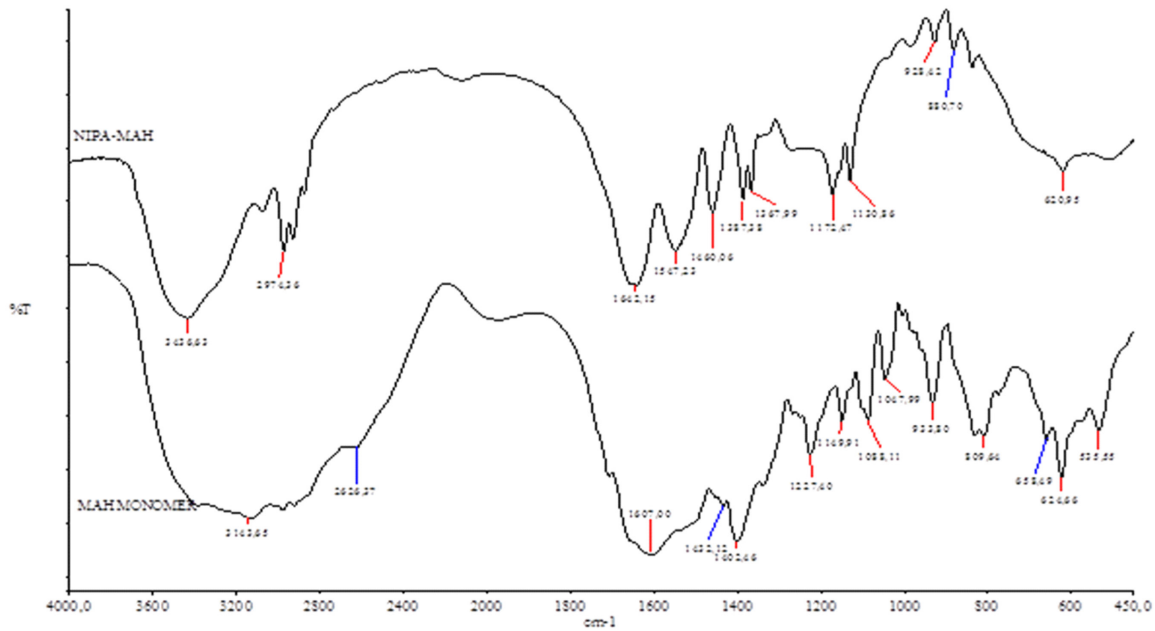

Figure 3. FT-IR spectrum of the poly(NIPA-MAH) cryogel (on top) as compared to that of the MAH monomer (at the bottom).

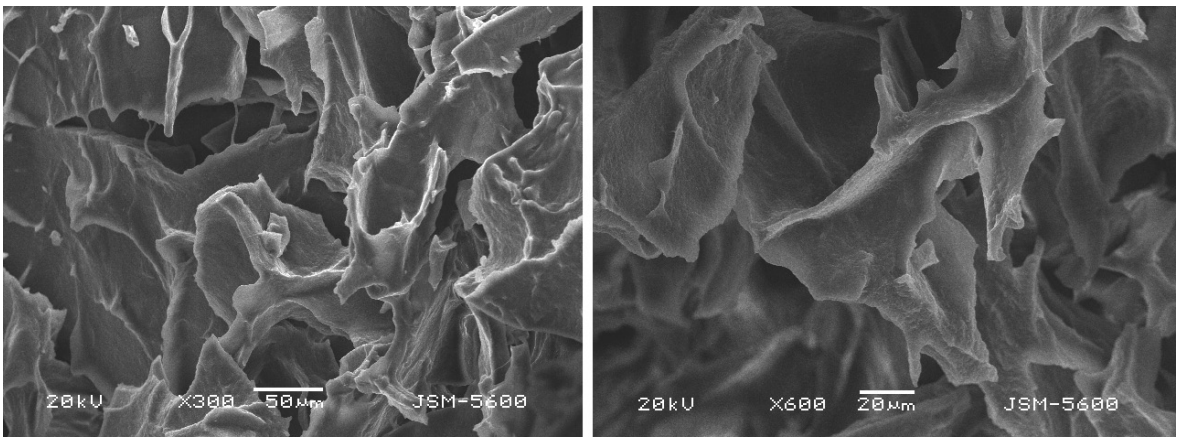

Figure 4. Scanning electron micrographs of the poly(NIPA-MAH) cryogel magnified by 300 times (A), and 600 times (B).

(above LCST), Figure 2A, and at 19ºC (belove LCST), Figure 2B, respectively. It was observed that the poly(NIPA-MAH) cryogel shrank tightly in about 5 min in water at 49ºC, Figure 2A, strongly suggested that the surface structure of the cryogel adopts a very hydrophobic nature at temperatures above LCST. In another set of experiments, the cryogel swelled rapidly and equilibrated in about 5 min in water at 19ºC, Figure 2B, with a considerably high maximum swelling ratio of 2208% and a water-uptake capacity of 22.08 g H_2O/g dry polymer, suggesting that the poly(NIPA-MAH) cryogel is highly hydrophilic at temperatures below LCST.

Figure 3 compares the FT-IR spectra for the poly(NIPA-MAH) cryogel, the spectrum on top, and MAH monomer, the spectrum at the bottom. The characteristic FT-IR peaks for the poly(NIPA-MAH) cryogel are seen at 1367 cm^{-1} and 1387 cm^{-1} C-H stretching broad band of -$CH(CH_3)_2$ at around 3436 cm^{-1} indicates the N-H stretching vibration of secondary amide in Figure 3, The bands at around 2974 cm^{-1} and 2873 cm^{-1} are assigned to asymmetric and symmetric C-H stretching vibrations, respectively of NIPA. The appearance of strong bands at around frequencies of 1642 cm^{-1} (C=O-NH) and 1538 cm^{-1} (N-H bending, amide II) indicate the incorporation of MAH monomer into the polymer structure.

The scanning electron micrographs of the poly(NIPA-MAH) cryogel taken at 25ºC and magnified by 300 and 600 times are shown in Figures 4A and 4B, respectively, in which highly interconnected macroporous cryogel structure is clearly seen. The large pores in the cryogel, in a sence, are useful in enabling easy flow of biomolecules in viscous fluids such as blood and plasma samples. It was determined that fast protein liquid chromatography columns packed with the dual-featured poly(NIPA-MAH) cryogel material were able to efficiently separate biomolecules out of blood samples at temperatures above LCST.

CONCLUSION

Elastic, spongy, and mechanically stable thermosensitive monolithic poly(NIPA-MAH) cryogels with 0.8 cm in diameter and 4.0 cm in length were synthesized and characterized. The cryogels are cheap materials and they can be used both as reusable materials and also offer great advantages when studying with viscous media [34-39]. The megaporous structure of cryogels allows direct processing of whole blood. The mega porous structures provide rapid response to stimuli as compared to classic gels besides of their low flow resistance. When the temperature changed, the properties of the surface of the poly(NIPA-MAH) cryogel switched from hydrophilic to hydrophobic. The copolymer was temperature sensitive. The poly(NIPA-MAH) cryogel present potentially attractive biotechnological field, as it has changeable surface. It appears that the poly(NIPA-MAH) cryogels can well be applied for the separation and purification of biomolecules and metal ions.

We expect that our characterization studies could help chemists as well as non-chemists in understanding the structural changes of termosensitive porous polymeric cryogels systems.

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