Preparation and Characterization of Pullulan/Poly(N-vinylimidazole) Gels

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ABSTRACT

Pullulan/Poly(N-vinylimidazole) (PNVI) hybrid materials have been prepared and characterized according to their physicochemical properties. Graft copolymerization and cryopolymerization/cryogelation approaches were applied in the preparation of pullulan/poly(N-vinylimidazole) samples.

Graft copolymerization of PNVI onto pullulan backbone was studied in aqueous solution by using ammonium persulphate initiation. A maximum grafting yield of 513% was obtained using 8.00 g/L pullulan, 0.66 M NVI and 0.18 M ammonium persulphate at 40°C for 3 h reaction duration conditions. Water soluble pullulan-*graft*-PNVI samples were obtained that exhibited antibacterial and antifungal activities. These samples precipitate out of solution via complex formation with polyanions such as tripolyphosphate and citrate ions.

Epichlorohydrin (ECH) crosslinked pullulan gel was used as substrate for grafting of PNVI to obtain pullulan/PNVI hybrid gels. The maximum grafting yield obtained for pullulan-ECH-*graft*-PNVI samples was 189% when 8.0 g/L pullulan gel, 0.57 M NVI and 0.18 M ammonium persulphate were used at 40°C for 3 h reaction duration conditions. Lower grafting yields were obtained due to the heterogeneous nature of the reaction medium when compared to the grafting yields in solution. Pullulan-ECH-*graft*-PNVI exhibit superabsorbency within 700-1300% and 6000-8500% ranges equilibrium water retention capacity in distilled water and in aqueous acidic medium respectively.

Cryogels of pullulan in the presence of ECH, PNVI cryogels in the presence and in the absence of ECH and pullulan/PNVI cryogels in the presence of ECH were prepared. Product yields and gel fraction yields were determined and correlated with cryogel preparation conditions. Similar to pullulan-ECH-*graft*-PNVI samples, the cryogels acted as hydrogels with maximum equilibrium swelling capacities of 425%, 240%, 270% and 620%, for pullulan-ECH, PNVI, PNVI-ECH, and pullulan-ECH-PNVI cryogel samples, respectively.

The products were characterized by elemental analysis, FTIR-ATR spectrometry, H-1 NMR, XRD, TGA and SEM analyses. Physicochemical properties were related to sample structure and preparation conditions.

Keywords: pullulan, poly(N-vinylimidazole), graft copolymer, cryogel, hydrogel

ÖZ

Bu tez çalışmasının konusu pulular/poly(N-vinylimidazole) hibrit malzemelerin hazırlanması ve fizikokimyasal özelliklerinin belirlenmesidir. Bu malzemelerin hazırlanmasında aşı kopolimerizasyonu ve kriyopolimerleşme ve kriyojelleşme yöntemleri kullanılmıştır. Homojen ortamda, sulu çözeltide amonyum persülfat başlatıcı varlığında hazırlanan aşı kopolimerlerin için en yüksek aşılanma yüzdesi 8.00 g/L pullulan, 0.66 M NVI ve 0.18 M ammonium persulphate, 40°C, 3 saat koşullarında, 513% olarak bulunmuştur. Bu örneklerin suda çözündükleri, antibakteriyal ve antifungal aktiviteye sahip oldukları ve polianyonlarla kompleks yaparak çökeldikleri gözlemlenmiştir.

Epichlorohydrin ile çapraz bağlanmaya uğratılarak elde edilmiş olan pulular-ECH jeller ise heterojen ortamda poly(N-vinylimidazole) ile aşılanmış ve en yüksek 189%, 8.0 g/L pullulan jel, 0.57 M NVI ve 0.18 M ammonium persulphate, 40°C, 3 saat, aşılanma yüzdesine ulaşılmıştır. Ortamın heterojen olması nedeniyle aşılanma yüzdeleri çözelti içinde elde edilen kopolimerlere göre daha düşüktür. Bu örnekler sulu ortamda süperabsorban hidrojel davranışı göstererek saf su içinde %700-%1300 ve asitli, sulu ortamda %6000-%8500 aralığında su tutma kapasitesine sahip olduklarını göstermişlerdir.

Pulular-ECH, PNVI, PNVI-ECH ve pulular-ECH-PNVI kriyojeller elde edilmiş ve ürün yüzdeleri ile jel fraksiyonları kriyojel hazırlama koşullarına göre analiz edilmiştir. Bu jellerin de hidrojel davranışı gösterdikleri ve sırasıyla pulular-ECH,

PNVI, PNVI-ECH ve pululan-ECH-PNVI kriyojellerin en yüksek su tutma kapasitelerinin dengede 425%, 240%, 270% ve 620% olduğu gözlemlenmiştir.

Örnekler, elemental analiz, FTIR-ATR, H-1 NMR, TGA, XRD ve SEM analiz yöntemleri ile karaktarize edlmişlerdir. Fizikokimyasal özellikler, örneklerin hazırlnama koşulları ve yapılarına göre değerlendirilmiştir.

Anahtar Kelimeler: pullulan, poly(N-vinylimidazole), aşı kopolymer, kriyojel, hidrojel

DEDICATION

Dedicate to my family…

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Chapter 1

INTRODUCTION

The subject of this thesis is the synthesis of pullulan/PNVI hybrid materials. Graft copolymerization and cryopolymerization/cryogelation approaches were applied to prepare pullulan/poly(N-vinylimidazole) samples. The aim is to modify pullulan, a water soluble nontoxic polysaccharide, by inserting NVI moieties into its structure, and blend useful characteristics of NVI such as cationic nature, antibacterial and antifungal activity to pullulan. The products are anticipated to have biomedical and environmental applications. The thesis does not only describe the synthesis and characterization of pullulan/PNVI hybrid materials, but also provides analysis of structure-property relationships of the products synthesized. Pullulan/PNVI graft copolymers or cryogels are new natural/synthetic hybrid polymeric materials originally reported within the scope of this thesis.

The first chapter of the thesis gives a brief introduction on the theoretical background of the work.

Graft copolymerization was carried out in solution and under heterogeneous conditions. Pullulan and NVI are two compatible chemicals, which are soluble in water. Therefore, graft copolymerization of PNVI onto pullulan can be carried out in aqueous medium using a water-soluble initiator namely ammonium persulphate (APS). Free radical polymerization mechanism operates in grafting of PNVI onto

pullulan via APS initiation. Pullulan-*graft*-PNVI samples were synthesized by changing the preparation conditions such as pullulan concentration, NVI concentration, APS concentration, reaction time and reaction temperature. Optimization of synthesis conditions and investigation of the physicochemical properties of these samples constitute the second chapter of this thesis.

In addition to synthesizing graft copolymers in solution, heterogeneous grafting conditions were also tested to obtain PNVI grafted pullulan gels. Pullulan was crosslinked with ECH and grafting reaction was carried out on the crosslinked pullulan gels by APS initiation. The third chapter explains the synthesis and properties of pullulan-ECH-*graft*-PNVI gels.

In the fourth chapter, cryogelation and cryopolymerization approaches are discussed for the synthesis of pullulan and PNVI based cryogels.

Pullulan-*graft*-PNVI, pulluan-ECH-*graft*-PNVI, pullulan-ECH cryogels, and pullulan-ECH-PNVI cryogels have been synthesized and reported for the first time by the author of the thesis. Furthermore, there are no reports in the literature regarding synthesis and characterization of PNVI cryogels alone, as given in this thesis. It should be mentioned that grafting study in solution was initialized during the author's master's studies.

1.1 Graft Copolymerization

Polymer modification is needed to produce materials with desired properties. There are various methods of polymer modification. Graft copolymerization is one of the versatile methods to modify polymers and prepare novel materials [1]. Representation scheme of a monomer grafting onto a polymer is shown in Figure 1.1.

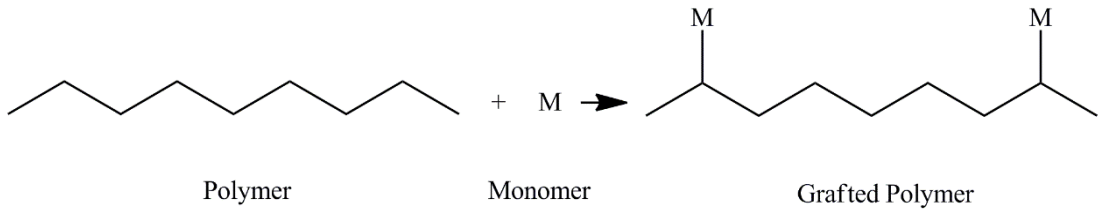

Figure 1.1. Representation structure for polymer grafting

A widely used grafting method onto polymers is free radical polymerization. In order to form active sites (free radicals) in the reaction medium, a suitable free radical initiator is required to initiate the polymerization reaction via radicals available in the medium. There are various direct and indirect free radical initiators. One of the most popular free radical initiator is persulphate compound such as ammonium persulphate and potassium persulphate. Persulphate initiators are widely used in different studies either as a direct or an indirect initiator [1-6]. Persulphate initiation is shown in Figure 1.2.

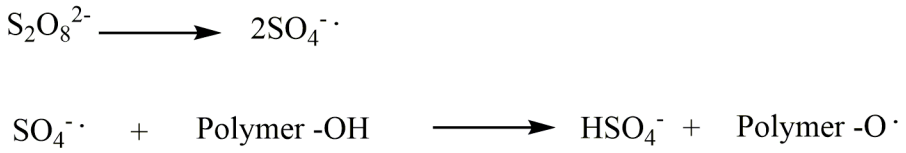

Figure 1.2. Persulphate initiation as a free radical initiator

Grafting has different parameters such as grafting yield (G%), and homopolymerization yield (H%). Optimization of grafting parameters are possible in terms of monomer concentration, substrate concentration, initiator concentration, reaction temperature and reaction duration by using Equation (2.1) and (2.2). In addition, the effect of any crosslinking agent or any other reaction condition such as acidity may need to be investigated [7].

1.2 Hydrogels

Hydrogels are three dimensional crosslinked polymer networks having high swelling capacity. Hydrophilic groups of the hydrogel network give absorption capability to the gels. Hydrogels are able to absorb and retain a great amounts of water. The gel rigidity is specified by the amount of imprisoned liquid in the polymer network. The gelation may go through chemical or physical crosslinking reactions [8-11].

Chemical gels are permanent gels having covalently crosslinked networks. In order to prepare a crosslinked network, a crosslinking agent is required. The crosslinking agent helps the backbone and monomer to attach through strong and stable covalent bonds. Physical gels are named as reversible gels. Physically crosslinked gels network interact by secondary forces such as hydrogen bonding, ionic and hydrophobic interactions or the networks entanglement brings molecules together.

Hydrogels may have some response to a certain stimuli and they may be thermo responsive, light responsive, electric responsive, pH responsive or chemically responsive polymer gels. Applications of hydrogels span in various fields such as civil engineering, electric electronic engineering, agriculture, medical, biotechnology, chemical processing, and food industry [11-13]. Some examples of hydrogels are poly(ethylene glycol) hydrogels modified for tissue engineering and drug delivery [14,15], pullulan based gels with various applications such as tissue engineering [16] and drug delivery for tumor extracellular in human body [17], poly(vinylimidazole) hydrogel initiated by using UV irradiation [18] for heavy metals removal application [19].

1.3 Cryogels

Cryogels are prepared under a freezing condition in an aqueous medium solution. The solvent act as porogen during the polymerization of the gel. Having a simple and cheap preparation method in compare to other kinds of gels make cryogels cheaper than any kind of polymer gels [20-24].

Cryogels are a kind of hydrophilic 3D polymer networks connected by crosslink bonds and contain interconnected large pores in the polymeric network. Due to hydrophilic sides in their networks, cryogels are able to swell by absorbing great amount of water. The pores size range of cryogels is reported as 10-200 μm [20]. Cryogels have different applications in industry such as bioprocessing application, tissue engineering, drug delivery, microbiology and water treatment [20-22]. As an example for bioengineering multi proposed cryogel, agarose–alginate is prepared by Anuj Tripathi [25] and poly(vinyl alcohol) cryogels are prepared for biomedical applications [26].

1.4 Pullulan

Pullulan is a linear fungal polysaccharide made up of α-1,4-linked maltotriose units, linked by α-1,6-glycosidic bonds. It was discovered by Bauer in 1938 and was first examined by Bender who named it as pullulan. It is produced by fungal fermentation of starch by *Aureobasidium pullulans (A. pullulans).* Pullulan is a degradable polysaccharide with molecular formula $(C_6H_{12}O_5)_n$ and 100,000-200,000 molecular weight range [16]. The chemical structure of pullulan is shown in Figure 1.3. Pullulan is a water soluble, adhesive polymer with hydrogel, film and fiber forming properties [27-30].

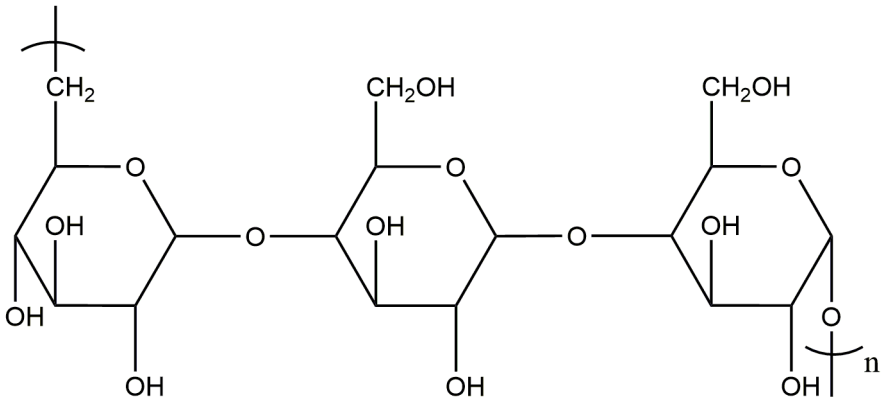

Figure 1.3. The maltotriose-repeating units of pullulan

1.4.1 Applications of Pullulan

Pullulan and its derivatives are promising materials for biomedical applications [29]. In the biomedical field, applications of the pullulan and modified pullulans range from drug delivery and gene delivery to tissue engineering, vaccination, and medical imaging [29] owing to the nontoxicity, biodegradability and versatile physical and chemical traits of the polymer. It can be used for capsule coating due to its film forming ability and adhesiveness. It can form microparticles, nanoparticles, nanogels and films for targeted drug delivery. Pullulan based scaffolds exhibit appropriate mechanical properties, water retention capacity and biocompatibility allowing cell adhesion and cell growth [29]. Pullulan is a neutral polysaccharide and has a potential to be used as plasma expander similar to dextran. Pullulan films are excellent food packaging materials as they are mechanically strong, impermeable to oxygen, and nontoxic [30]. Pullulan is a food and cosmetics additive, which acts as binder and stabilizer in many formulations [27].

1.4.2 Modified Pullulans

Chemical modification of pullulan is possible via functional –OH groups available on the polymer backbone. Several different types of modification reactions such as carboxylation and sulfation, were carried out on the polysaccharide with the aim of

obtaining negatively charged pullulans that will act as drug conjugates or as blood coagulant similar to heparin [27]. Oxidation of the pyranose ring brings in further functionality together with increasing the flexibility of the chain due to ring opening as a result of oxidation. Hydrophobicity may be imparted on the polymer via cholesterylation [27]. Derivatives of pullulan such as pullulan acetate, carboxymethyl pullulan, pullulan succinylate, cholesterylpullulan and polyethyleneimine pullulan have been proposed as drug and gene carriers [27].

1.5 N-vinylimidazole (NVI)

N-vinylimidazole is a vinyl monomer by $C_5H_6N_2$ chemical formula. It has molar mass of 94.1145 g/mol. This molecule also named as 1-ethenyl vinyl imidazole, 1-vinyl imidazole and 1H imidazole. N-vinylimidazole exists as a yellow color liquid and it is freely water soluble [31]. The chemical structure of N-vinyl imidazole (NVI) monomer is shown in Figure 1.4.

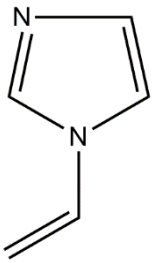

Figure 1.4. N-vinylimidazole molecule structure

N-vinylimidazole monomer has antibacterial and antifungal activity due to having imidazole ring on its structure [19,20]. N-vinylimidazole monomer is able to polymerize similarly to other vinyl monomers by using a suitable initiator [32].

1.5.1 Poly(N-vinylimidazole)

N-vinylimidazole (NVI) monomer may polymerize through free radical polymerization or ionic polymerization to form poly(N-vinylimidazole) (PNVI) [33].

Not only the monomer but also its polymer has antibacterial and antifungal properties due to the amine groups on the polymer backbone [32]. PNVI is shown in Figure 1.5, is a water soluble and biocompatible polymer which can act as a polycation in acid solution and is capable to form hydrogels via crosslinking [14].

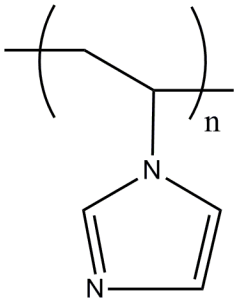

Figure 1.5. PNVI structure

1.5.2 Poly(N-vinylimidazole Grafted Polymers and Applications

Poly(N-vinylimidazole) grafted polymers have demonstrated useful biorelated properties. For example, silicon rubber [15], poly(vinylchloride) [16], carboxymethyl starch [17] and chitosan [18] exhibited antibacterial activity. Hyaluronic acid-*graft*-poly(N-vinylimidazole) has shown antitumor activity [19]. Pullulan and dextran that were initially modified by poly(ethyleneimine) were further grafted by vinyl imidazole to produce potential gene delivery vectors with reduced cytotoxicity and improved transfection efficiency [20]. In addition to their biomedical applications poly(N-vinylimidazole) grafted polymers are potential adsorbents for heavy metal removal from solution [21].

Chapter 2

GRAFTING OF POLY(N-VINYLIMIDAZOLE) ONTO PULLULAN IN SOLUTION

Grafting of vinyl polymers onto pullulan was generally performed via free radical or redox initiated polymerization. Several examples are available including grafting of poly(ethylene glycol) [41], methyl acrylate [6], poly(methyl methacrylate) [7], 3-acrylamidopropyl trimethylammonium chloride [8], N-isopropylacrylamide [9] and polyacrylamide [43]. Some pullulan-*graft*-PEG polymer samples obtained were not only soluble in water and DMSO but also in methanol [41]. Water absorbing capacity of pullulan-*graft*-poly(methyl acrylate) polymer was found to decrease by increasing grafting yield [6]. Methyl acrylate grafting reduced the hydrophilic character of pullulan. Pullulan-*graft*-poly(methyl methacrylate) copolymer was obtained as nanoparticles by atom transfer radical polymerization [7]. Grafting of 3-acrylamidopropyl trimethylammonium chloride onto pullulan is reported as a free radical polymerization reaction to design a polymer to be used in drug delivery and waste water treatment processes [8]. Indomethacin loaded pullulan-*graft*-poly(N-isopropylacrylamide) nano particles demonstrated controlled release of the drug [44]. Pullulan derivatives carrying cationic charge are especially needed for DNA complexation and gene delivery [11]. Cationized pullulans for gene delivery have been prepared by diethyl amino ethyl (DEAE) modification. Further crosslinking with phosphorus oxychloride gave tubular hydrogels of DEAE pullulan with high affinity

for DNA [12]. Polyethyleneimine (PEI) grafted pullulans are highly promising gene carriers due to the high cationic density of PEI-pullulan copolymer [13].

The first part of this thesis work explained below presents synthesis of pullulan-*graft*-PNVI copolymers in solution. The obtained copolymers were characterized and tested for their antibacterial and antifungal activities.

2.1 Experimental

2.1.1 Material

Pullulan (Hayashibara, Japan), N-vinyl imidazole (Aldrich, Germany), ammonium persulphate (Sigma Aldrich, Germany), cerium ammonium nitrate (Aldrich, Germany), sodium citrate (Sigma, Germany), pentasodium tripolyphosphate (Sigma Aldrich, Germany), hydrochloric acid (Sigma, Germany), ethanol (SAFA, North Cyprus), acetone (TEKIM, North Cyprus) were used without any further purification.

2.1.2 Synthesis of Pullulan-*graft*-PNVI under Homogenous Condition

Weighed amount of pullulan was dissolved in 25 mL of distilled water under magnetic stirring to obtain a homogenous solution. N-vinylimidazole (NVI) and the initiator, ammonium persulphate (APS), were added into the solution under nitrogen atmosphere and the reaction was carried out at constant temperature under magnetic stirring for a given period of time as shown in Figure 2.1. The preparation conditions are given in Table 2.1. Then, the solution was poured into acetone with vigorous stirring to precipitate the product. The precipitate was filtered, washed with ethanol to remove any homopolymer formed. Two experimental runs were carried out for each sample, and grafting yields were reported as the average of two samples with ±5% error. Grafting yield (G%), and homopolymer yield (H%) were calculated by using Equation (2.1) and (2.2) respectively as follows:

$$G\% = \frac{W_2 - W_0}{W_0} \times 100\% \quad (2.1)$$

$$H\% = \frac{W_1 - W_2}{W_3} \times 100\% \quad (2.2)$$

Grafting yields by elemental analysis are also calculated by considering nitrogen percentage obtained from elemental analysis as given in Equation (2.3).

$$G_E\% = \frac{W_4}{W_0} \times 100\% \quad (2.3)$$

where,

W_0=initial weight of pullulan (g), W_1=weight of grafted material before washing process (g), W_2=weight of grafted material after washing process (g), W_3=initial weight of monomer (NVI) (g), W_4= weight of monomer (NVI) in grafted product from elemental analysis (g).

Figure 2.1. The grafting reaction preparation under nitrogen atmosphere

Table 2.1. Grafting conditions, grafting yields and homopolymer yields for pullulan-*graft*-PNVI

No	[Pullulan] (g/L)	[NVI] M	[APS] M	Time (h)	Temp (°C)	G%	H%
1	8.00	0.09	0.18	3	40	46.0	23
2	8.00	0.22	0.18	3	40	195	4.0
3	8.00	0.31	0.18	3	40	140	14
4	8.00	0.44	0.18	3	40	320	6.3
5	8.00	0.66	0.18	3	40	513	3.5
6	8.00	0.75	0.18	3	40	493	6.5
7	8.00	0.22	0.18	1	40	96.0	3.0
8	8.00	0.22	0.18	2	40	131	4.8
9	8.00	0.22	0.18	4	40	189	5.5
10	8.00	0.22	0.18	3	30	60.0	6.0
11	8.00	0.22	0.18	3	50	202	10
12	8.00	0.22	0.087	3	40	89.0	8.0
13	8.00	0.22	0.26	3	40	200	15
14	20.0	0.22	0.18	3	40	61.0	8.0
15	40.5	0.22	0.18	3	40	50.0	20

2.2 Characterization Studies

FTIR-ATR analysis was carried out using Perkin Elmer Spectrum-Two in Eastern Mediterranean University, North Cyprus. Elemental analysis of pullulan and grafted samples was carried out at Central Laboratory of METU in Ankara using LECO, CHNS-932 instrument. H-1 NMR analysis of pullulan and grafted samples was carried out at Central Laboratory of METU in Ankara using Bruker Biospin Ultrashield TM 300 MHz instrument. Thermal Gravimetric Analysis (TGA) analysis of pullulan and grafted samples was carried out at Central Laboratory of METU using Perkin Elmer Pyris 1 instrument in Ankara. The samples were heated at a rate of 10°C/min under nitrogen atmosphere. Size Exclusion Chromatography (SEC) analysis of samples was carried out in Hacettepe University, Ankara Turkey. Waters-Breeze SEC and 2000-1000-500-250 ultrahydrogel columns were used for molecular weight analysis and a primary calibration curve was constructed by using narrow molecular weight pullulan

standards obtained from Shodex Company. NaCl (0.1 M) was used as the eluting solvent. Antifungal and antimicrobial analysis were carried out in Medicine department of Eastern Mediterranean University, North Cyprus.

The solubility of the samples was investigated in aqueous acid solution, in water and in DMSO. Each sample (0.05 g) was placed into either 20 mL of 0.10 M hydrochloric acid or 20 mL of distilled water or DMSO at room temperature.

For Complex Formation test, an aqueous solution of pentasodium tripolyphosphate was prepared by dissolving 0.50 g of the solid in water to obtain 10 mL solution. An aqueous sodium citrate solution was prepared in the same manner as the pentasodium tripolyphosphate solution. Pullulan-*graft*-PNVI (G%=513) sample was dissolved in 0.10 M hydrochloric acid solution to prepare 2.0 mL solution. In order to achieve complex formation between the tripolyphosphate ion and the grafted sample, 8.0 mL of the tripolyphosphate solution was added dropwise to 5.0 mL of the pullulan-*graft*-PNVI solution. Complex formation between the pentasodium citrate ions and pullulan-*graft*-PNVI was obtained by adding 4.0 mL of the citrate solution drop by drop into 5.0 mL of polymer solution.

2.3 Results and Discussion

Pullulan-*graft*-PNVI was synthesized in aqueous solution by dissolving pullulan, NVI, and ammonium persulphate (APS) in distilled water, under nitrogen atmosphere. Grafting conditions were optimized according to the monomer, pullulan and initiator concentrations, as well as grafting time and grafting temperature. It is anticipated that active free radical sites are created on the pullulan backbone by the action of the initiator onto which PNVI chains are grafted. Structural characterization has been

carried out by FTIR-ATR and H-1 NMR spectroscopies. The most likely chemical structure of pullulan-*graft*-PNVI is shown in Figure 2.2.

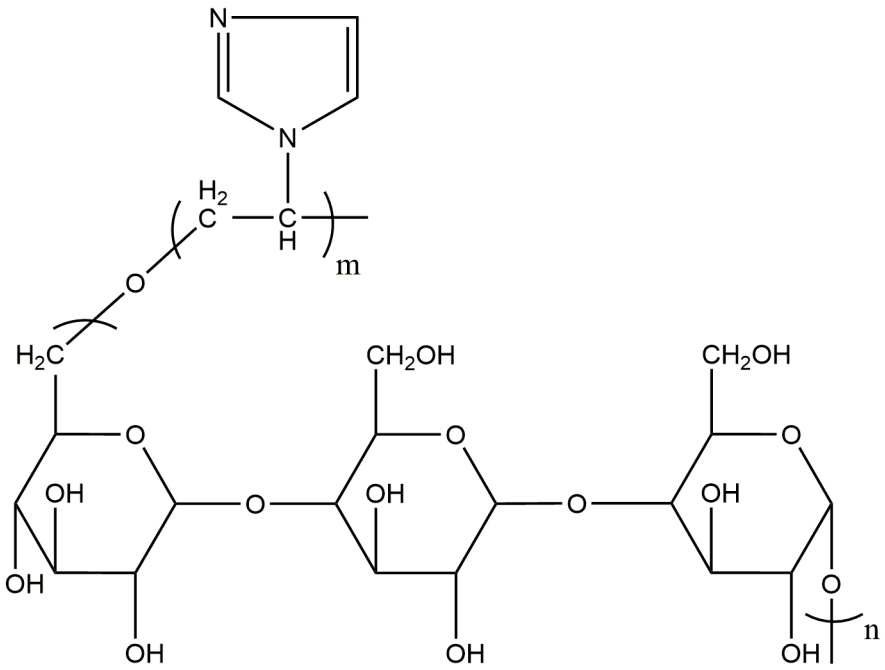

Figure 2.2. Pullulan-*graft*-PNVI structure

Table 2.1 shows the grafting yields and homopolymer yields obtained under the conditions studied. The highest grafting yield (G%=513) is obtained by 8.00 g/L of pullulan, 0.66 M of N-vinylimidazole (NVI) and 0.18 M of ammonium persulphate at 40°C in 3 h. Homopolymerization percentages changing in between 3%-23% were calculated. The H% presented in Table 2.1 showed a decreasing trend relative to the grafting yield. The effect of each variable on G% is shown separately in Figure 2. (3-7).

2.3.1 Effect of Grafting Conditions on Grafting Yields under Homogenous Condition

The effect of monomer concentration, pullulan concentration, initiator concentration, time and temperature on the grafting yield has been investigated. The highest grafting yield (513%) of pullulan-*graft*-PNVI was obtained using 8.00 g/L pullulan, 0.66 M

NVI, 0.18 M APS at 40 °C in aqueous solution for 3 h reaction duration under nitrogen atmosphere.

2.3.1.1 Effect of Monomer Concentration on Grafting Yield under Homogenous Condition

The grafting yield was optimized according to monomer concentration at 40°C reaction temperature, using 8.00 g/L pullulan, and 0.18 M ammonium persulphate (APS). The reaction was carried out for three hours in 25 mL solution in each case. The results are shown in Figure 2.3. It was observed that the grafting yield increased with increasing NVI concentration reaching the highest value (G%=513) using 0.66 M of the monomer. The grafting yield becomes constant upon further increase in the amount of the monomer to 0.75 M. After reaching a maximum value, further increase in the monomer concentration does not increase the grafting yield but there is a tendency to level off. A similar behavior was observed with grafting of PNVI onto chitosan [37]. The tendency to level off with NVI concentration can be attributed to possible degradative chain transfer reactions, which hinder further polymerization of NVI. Also steric hindrance due to the grafted chains on pullulan backbone may be another factor hindering any further grafting [36,37,43].

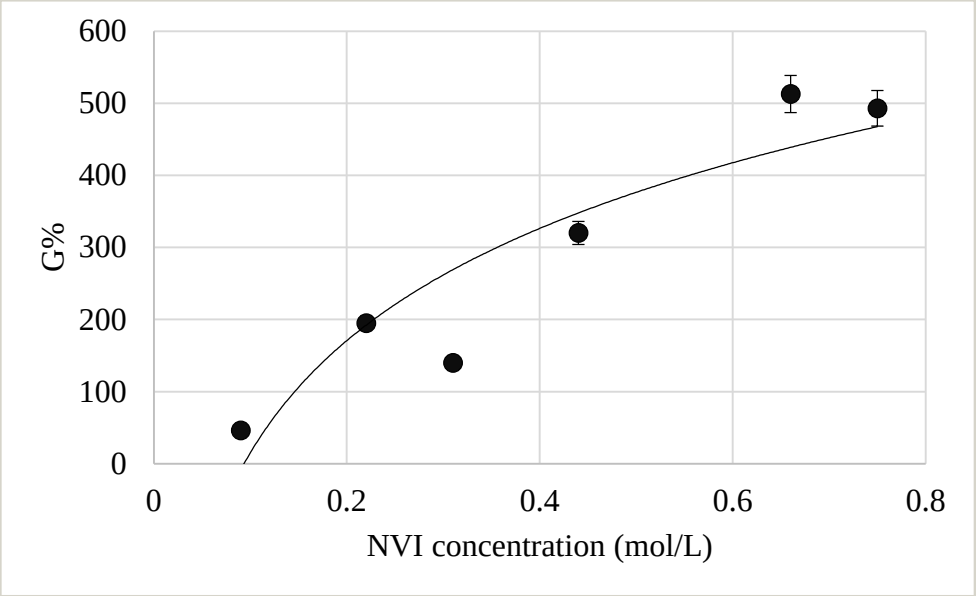

Figure 2.3. Effect of monomer concentration on grafting yield (G%) under homogenous condition

2.3.1.2 Effect of Pullulan Concentration on Grafting Yield under Homogenous Condition

By increasing pullulan concentration from 8.00 g/L to 20.0 g/L, G% decreases and reaches a minimum value when the amount of pullulan is in between 20.0 g/L-40.5 g/L as shown in Figure 2.4. A higher amount of pullulan provides higher number of possible sites available for activation by the initiating species. However, the number of active sites that can be created is limited by the amount of the initiating species, which is one factor limiting the grafting yield. Similarly, a lower pullulan/monomer ratio at higher amounts of pullulan results in lower grafting yields. Furthermore, solution viscosity increases at higher amounts of pullulan decreasing the mobility and diffusing ability of the reacting molecules [36,37].

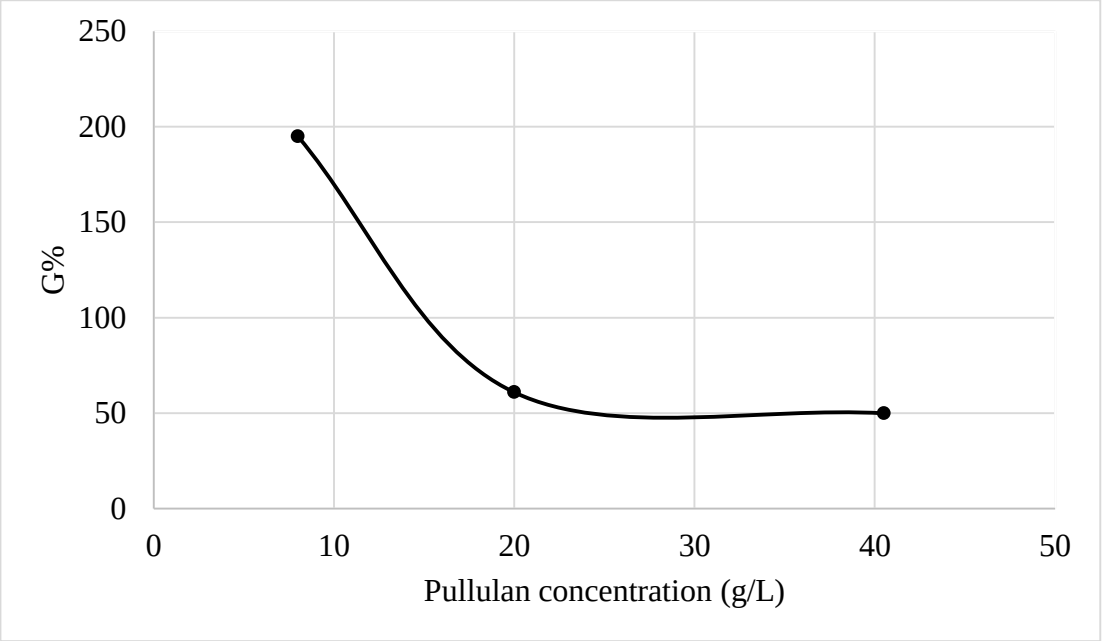

Figure 2.4. Effect of pullulan concentration on grafting yield under homogenous condition

2.3.1.3 Effect of Initiator Concentration on Grafting Yield under Homogenous Condition

In order to study the effect of the initiator concentration on the grafting yield of polymerization, various concentrations of initiator were used by keeping any other variable constant. Increasing the initiator concentration leads to a rapid increase in the grafting yields to a maximum value of 195% by using 0.18 M of initiator, and then grafting yield value becomes constant as can be followed from Figure 2.5. More free radicals due to a higher concentration of the initiator are expected to cause a higher amount of active sites on the pullulan backbone [36,37,43].

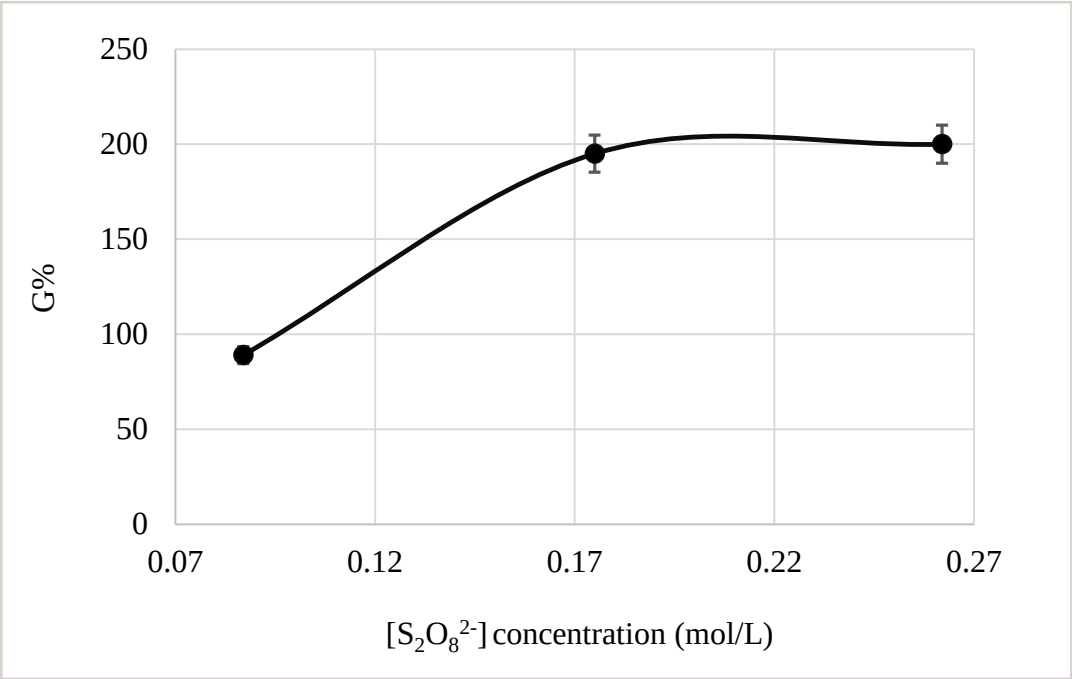

Figure 2.5. Effect of initiator concentration on grafting yield under homogenous condition

2.3.1.4 Effect of Reaction Duration on Grafting Yield under Homogenous Condition

According to Figure 2.6, the maximum grafting value reaches G%=200 in 3 hours. Afterwards, the grafting yields do not change with time due to the decrease in available monomer, initiator and grafting sites [36,37,43].

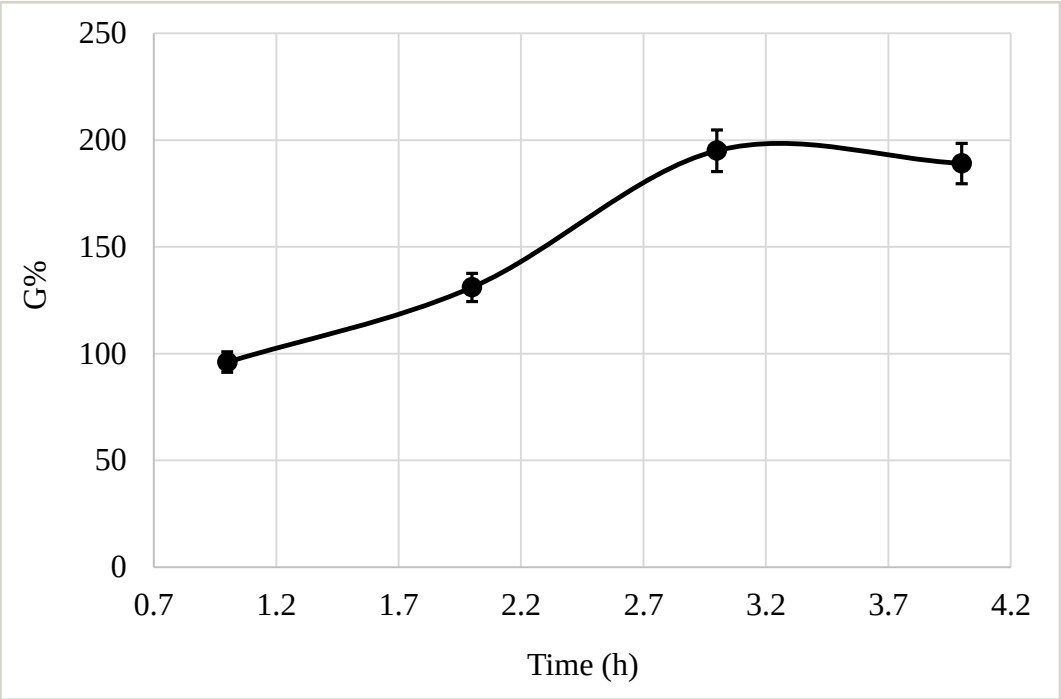

Figure 2.6. Effect of reaction duration on grafting yield under homogenous condition

2.3.1.5 Effect of Reaction Temperature on Grafting Yield under Homogenous Condition

Temperature effect was evaluated by changing temperature of the reaction from 30°C to 50°C. The maximum grafting percentage is reached at 40°C as G%= 200 as shown in Figure 2.7. The fact of increasing grafting yield by increasing temperature from 30°C to 40°C is the rate of reaction increases giving higher amount of grafted chains. Greater probability of chain transfer and chain termination reactions at higher temperatures together with decreased efficiency of the initiator can be considered as factors contributing to levelling off in the grafting yields [3,36,37,43].

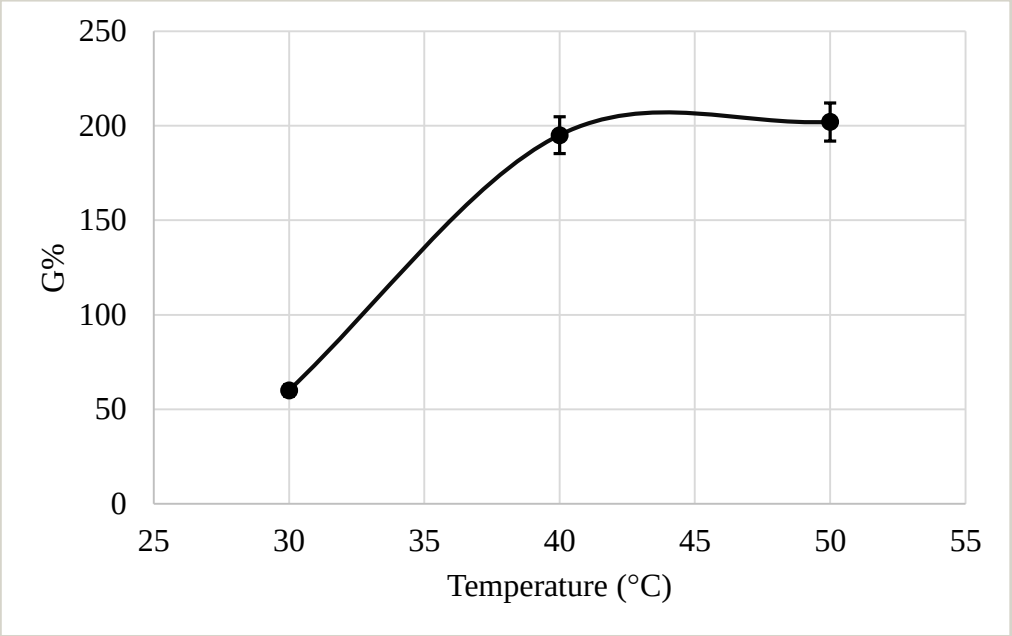

Figure 2.7. Effect of reaction temperature on grafting yield under homogenous condition

2.3.2 Elemental Analysis

Elemental analysis was carried out on the samples to find additional evidence for PNVI grafting. C%, H% and N% values of the samples obtained by increasing the monomer concentration at 40°C for 3 h of polymerization time using 8.00 g/L pullulan solution are reported in Table 2.2. It can be followed from the table that N% content of the samples increase with increasing G% value determined by gravimetric analysis, providing further evidence for imidazole grafting on the pullulan chain. The sample with lowest grafting yield (G%=46) has got the lowest nitrogen percentage (N%=0.70). The sample with the highest grafting yield (G%=513) bears the highest nitrogen percentage (N%=14.97). Furthermore, G_E% values were calculated according to N% values from elemental analysis, using Equation (2.3). The values agree with G% yields obtained by gravimetry within 22-26% except for sample 1. It is important to have imidazole ring grafted on pullulan to provide the neutral polysaccharide with cationic property in aqueous acid solution. Having cationic functionality brings in

complex formation capability with anionic polymers for many versatile applications such as gene delivery [39] and antimicrobial activity [35,37].

Table 2.2. Elemental analysis results of homogenous samples (N%, C%, H%), gravimetric grafting yield and elemental analysis grafting yield

Sample	[NVI] M	N% (Elemental Analysis)	C% (Elemental Analysis)	H% (Elemental Analysis)	G% (Gravimetric)	G_E% (Elemental Analysis)
Pullulan	-	-	39.16	6.38	-	-
1	0.09	0.70	20.98	5.89	46	11
2	0.22	12.68	19.78	5.68	195	255
3	0.31	12.54	19.55	5.56	140	190
4	0.44	14.53	20.01	5.69	320	438
5	0.66	14.97	19.27	5.43	513	662
6	0.75	-	-	-	493	-

2.3.3 FTIR-ATR Analysis

In Figure 2.8 (a) and (b) the FTIR-ATR spectrum of pullulan and pullulan-*graft*-PNVI (G%=513) samples are shown, respectively. In the FTIR-ATR spectrum of pullulan, at 3310 cm^{-1} the O-H stretching vibrations are observed. The C-H vibrations appear at 2930 cm^{-1} and the C-O stretching vibrations of the glycosidic and etheric bonds of the polymer are observed at 1148 cm^{-1}, 1078 cm^{-1}, 995 cm^{-1} and 929 cm^{-1}. The FTIR-ATR spectrum of PNVI homopolymer gives peaks at 3143 cm^{-1}, 2922 cm^{-1}, 2848 cm^{-1} are due to N-H stretching and C-H stretching vibrations, respectively. The stretching vibration of C=N, C-N and C=C of the imidazole ring appear at 1641 cm^{-1} and 1556 cm^{-1}. The C-H vibrations are observed at 1490 cm^{-1} and 1300 cm^{-1} region [44] . The spectra of the grafted products the characteristics of both pullulan and PNVI can be observed. In addition to glycosidic and etheric bond of pullulan in the region 1100-

900 cm^{-1}, characteristic C=N stretching vibrations of PNVI can be observed at 1450-1540-1570 cm^{-1} regions. The O-H stretching of the grafted sample is observed at lower wavenumbers due to the grafting reaction taking place on the -OH sites.

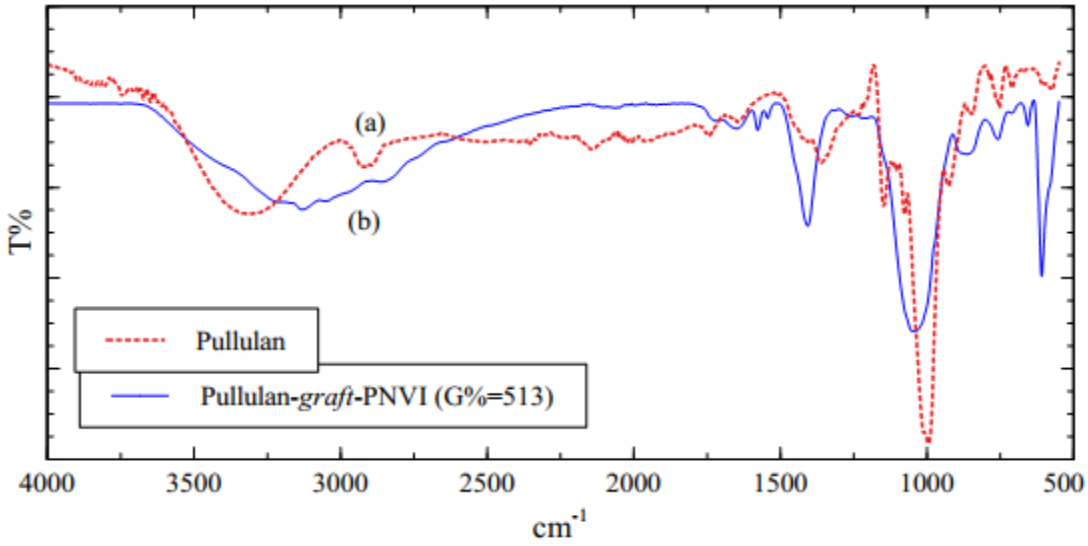

Figure 2.8. FTIR-ATR spectrum of (a) pullulan and (b) pullulan-*graft*-PNVI (G%=513) samples

2.3.4 H-1 NMR Analysis

NMR analysis of pullulan, Figure 2.9, shows peaks from 2.0 ppm to 5.5 ppm exhibiting hydrogens of the polysaccharide. While 1→6 and 1→4 anomeric protons are observed at 4.7 and 5.3 ppm, hydrogen atoms on C-2, C-3 and C-5 of each member of the maltotriose units are found at 3.0-4.0 ppm region. Methylene hydrogens on C-6, on the other hand, exhibit themselves at 1.8-2.5 ppm interval. Figure 2.10, the H-1 NMR spectrum of pullulan-*graft*-PNVI sample (G%=513) contains not only pullulan peaks but also peaks from vinylimidazole grafted on the polysaccharide at 7.6 ppm and 8.7 ppm. Furthermore, the increase in the fraction of methylene protons in the range 1.8-2.4 ppm interval according to the peak areas shown on the spectra, provides additional evidence of PNVI grafting on the pullulan backbone. The decrease in the peak area of

1→6 anomeric hydrogens at 5.3 ppm in the H-1 NMR spectrum of the grafted sample can be attributed to some chain degradation as revealed by GPC analysis.

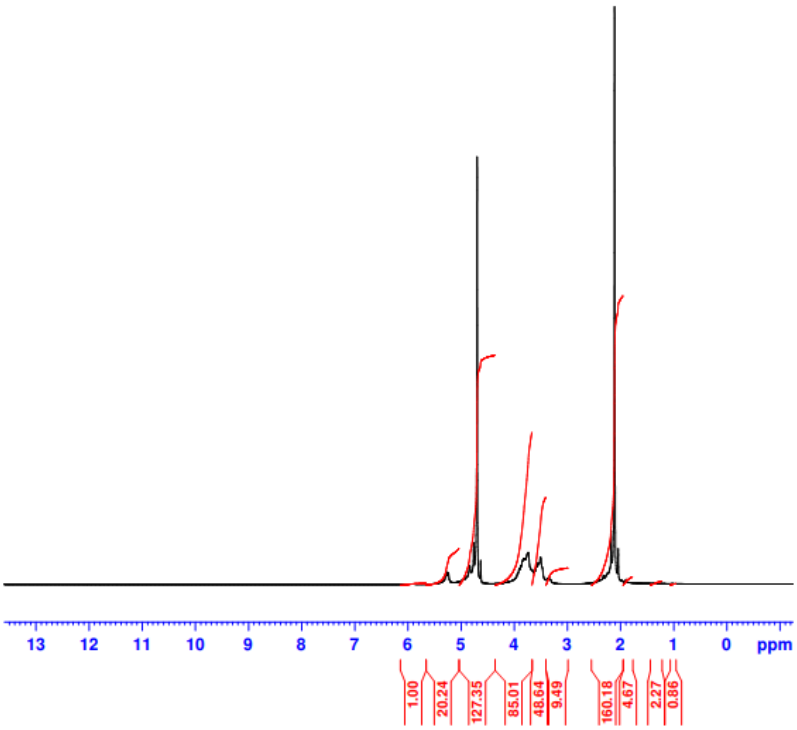

Figure 2.9. H-1 NMR spectrum of pullulan

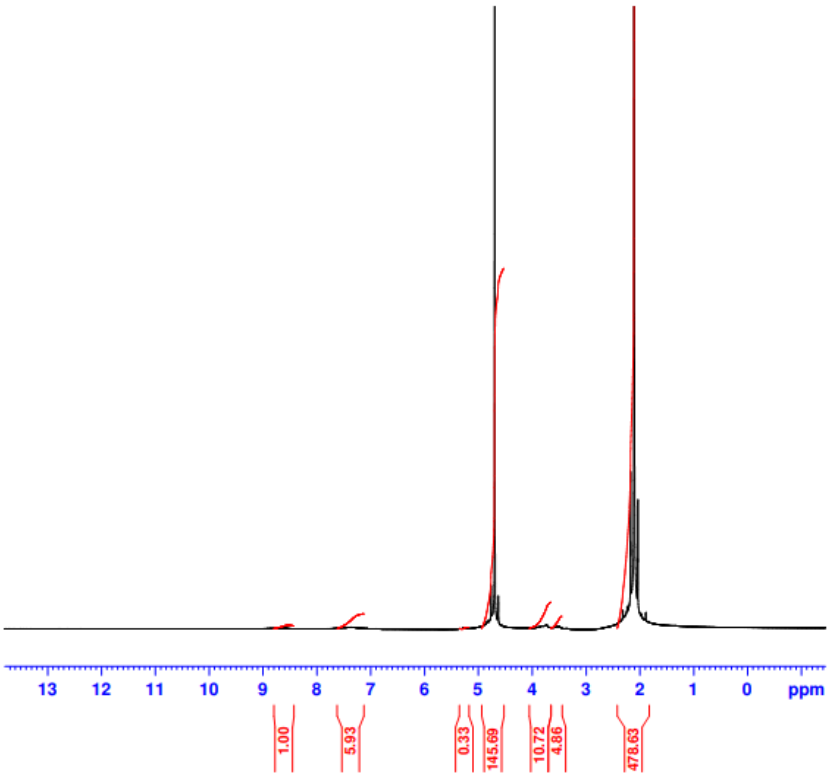

Figure 2.10. H-1 NMR spectrum of pullulan-*graft*-PNVI (G%=513)

2.3.5 TGA Analysis

TGA thermogram of pullulan and pullulan-*graft*-PNVI sample (G%=513), is given in Figure 2.11. Pullulan loses water up to 130°C. There is a 10% weight loss due to water loss. Similarly, pullulan-*graft*-PNVI loses water in the 25°C-160°C range with 10% weight loss. While pullulan decomposes sharply at 325°C to 20% of its original weight, the grafted sample decomposes in the same temperature range but at a slower rate than pullulan. After decomposition 25% of the grafted sample remains. Upon further heating pullulan completely decomposes at 600°C. Pullulan-*graft*-PNVI, on the other hand, retains 15% of its weight at 600°C. Hence, the grafted product has better thermal stability than pullulan.

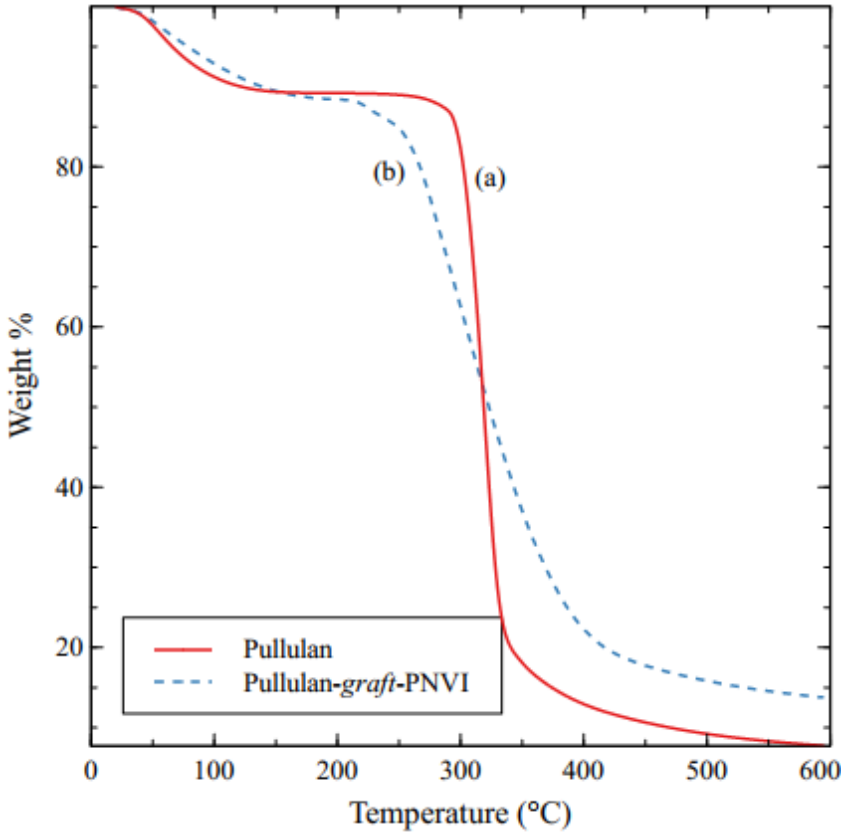

Figure 2.11. TGA curves of (a) pullulan, (b) pullulan-*graft*-PNVI

2.3.6 GPC Analysis (SEC)

Table 2.3 shows GPC for pullulan, pullulan-*graft*-PNVI (G%=42.7) and pullulan-*graft*-PNVI (G%=84.9). Pullulan as received from the producer has a molecular weight of 2.14×10^5 Da and a polydispersity index of 2.24 as determined by SEC. Two other samples, pullulan-*graft*-PNVI (G%=42.7) and pullulan-*graft*-PNVI (G%=84.9) treated with persulphate at 40°C for 2 h in the presence of NVI with the aim of grafting PNVI onto pullulan in homogenous conditions has molecular weight and PDI values of 1.32×10^5 and 1.95 and 4.4×10^4 and 1.95, respectively [46]. Increasing grafting percentage results in lower molecular weight values, Table 2.3.

Table 2.3. GPC analysis results for pullulan, pullulan-*graft*-PNVI (G%=42.7) and pullulan-*graft*-PNVI (G%=84.9)

Sample	**M_n (g/mol)**	**M_w (g/mol)**	**M_p**	**PDI**
Pullulan	154700	347000	214800	2.24
pullulan-*graft*-PNVI (G%=42.7)	92800	180700	132100	1.95
pullulan-*graft*-PNVI (G%=84.9)	26800	52400	44300	1.95

2.3.7 Solubility Tests

The products were tested for their solubility in distilled water, in 0.1 M hydrochloric acid solution and in DMSO. They are readily soluble in both pure water, in acidic solution and in DMSO.

2.3.8 Complex Formation

The nitrogen atom of the imidazole ring becomes protonated in acid solution, and hence produces complexes with polyanions. The optical pictures of the insoluble complex obtained upon mixing acidic pullulan-*graft*-PNVI with tripolyphosphate solution and sodium citrate solution are shown in Figure 2.12 and Figure 2.13, respectively. The opaque solutions obtained may further be freeze-dried to produce nanoparticles for further applications.

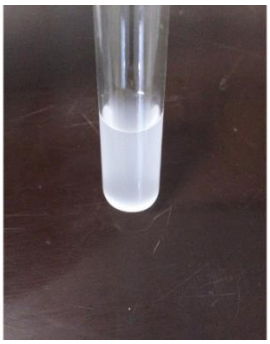

Figure 2.12. Optical picture of pullulan-*graft*-PNVI tripolyphosphate complex

Figure 2.13. Optical picture of pullulan-*graft*-PNVI citrate complex

2.3.9 Antimicrobial Activity of Homogenous Grafted Sample

Having amine group on N-vinylimidazole (NVI) monomer structure bears antimicrobial property [47], Figure 1.4. Pullulan is the backbone polymer of the grafted sample without having antimicrobial property. One available grafted sample (G%=89) was tested for antibacterial activity. Test has been done on two different bacteria, *S.aureus* bacteria and *E.coli* bacteria. The lowest concentration of antimicrobial agent (MIC) through antimicrobial test is found 20.025 mg/mL for S.aureus bacteria and 40.15 mg/mL for E.coli bacteria. The sample proved to be more affective on *S.aureus* bacteria than *E.coli* bacteria.

2.3.10 Antifungal Activity of Homogenous Grafted Sample

NVI monomer itself is an antifungal molecule due to having amine groups on its structure [48]. Pullulan is the backbone polymer without antifungal property.

Antifungal test was done against *Candida albicans* fungi on the samples given in Table 2.1 with different grafting yield. In order to do antifungal analysis same concentration (330 mg/mL) of each sample were prepared. Inhibition zones of NVI monomer and pullulan-*graft*-PNVI (G%=89) are shown in Figure 2.14 (a) and (b) respectively. While NVI monomer alone gives an inhibition zone diameter of 2.50 cm the grafted

product shows an inhibition zone diameter of 1.8 cm. The monomer has excellent antifungal activity which is diluted when present together with pullulan.

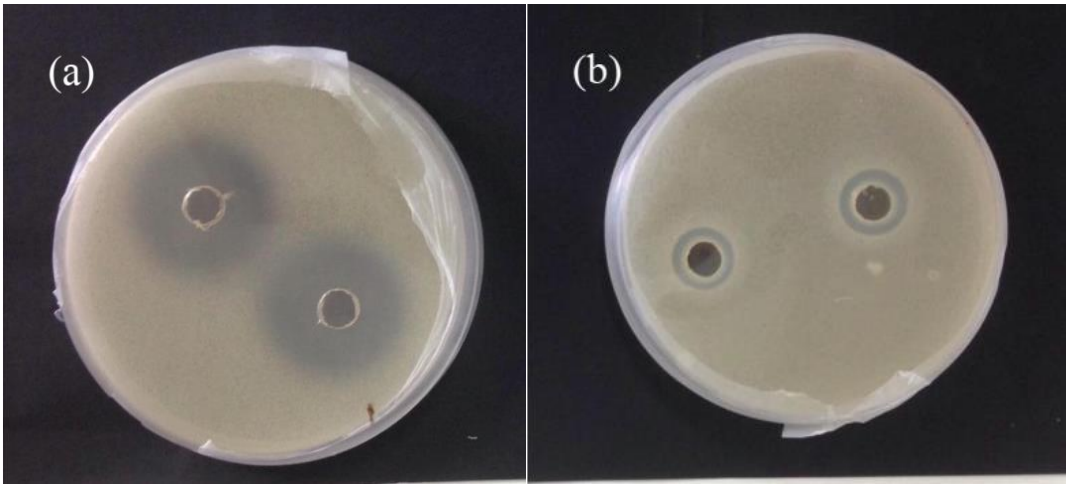

Figure 2.14. Antifungal analysis of (a) NVI monomer and (b) pullulan-*graft*-NVI sample 12 (G%=89.0) against *Candida albicans* fungi

Antifungal activities of grafted samples under same synthesis condition with grafting yields of (50%, 195%, 189%, 202%, 280% and 493%) are compared in Figure 2.15.Optical pictures of antifungal activity of samples having grafting percentage as 50%, 195%, 189%, 202%, 280% and 493% is shown in Figure 2.16. Accrording to Figure 2.15, no antifungal activity is observed up to 190% grafting yield. This may be attributed to simply not enough concentration of NVI units or inactivation of NVI units via H-bonding. When NVI is higher than a critical amount than not all units may find the correct orientation for H-bonding so they are free to act as an antifungal agent. Antifungal activity increases with increasing grafting yield at grafting values above the threshold value (190%). Increasing NVI content on the pullulan backbone results in better antifungal activity. Samples with higher grafting yields are expected to have lower molecular weights as exemplified in Table 2.3. Higher grafting also gives rise to higher branching, and lower viscosities. These factors contributed to easier diffusion of the polymer through the agar, leading to better antifungal property.

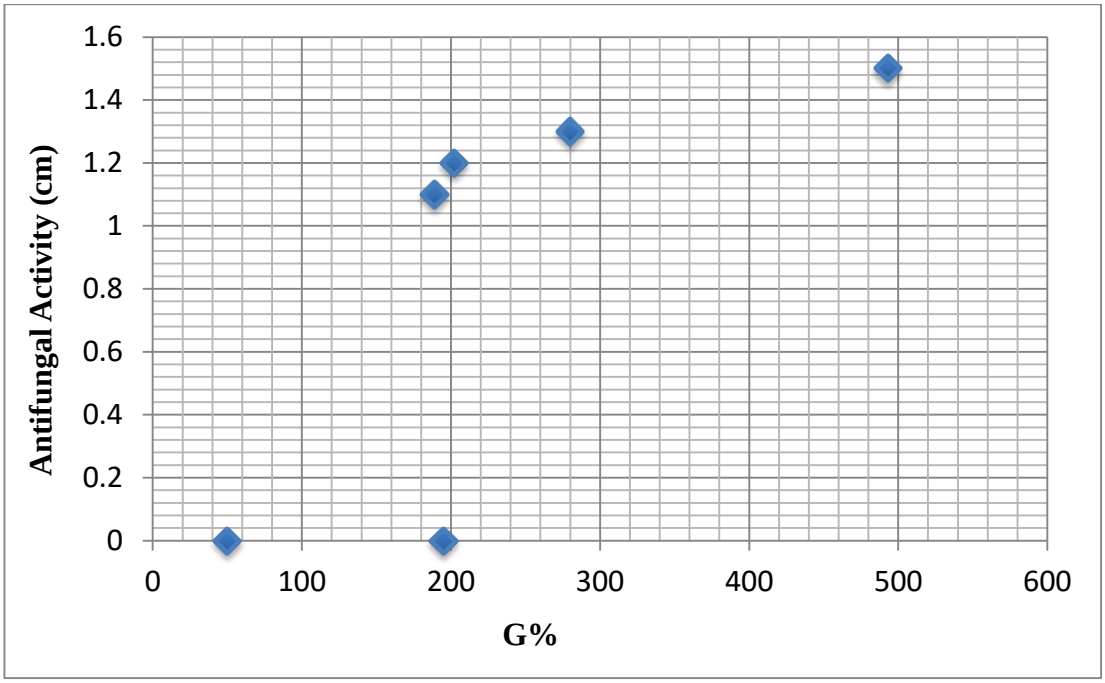

Figure 2.15. Effect of grafting yield on antifungal activity of samples against *Candida albicans* fungi

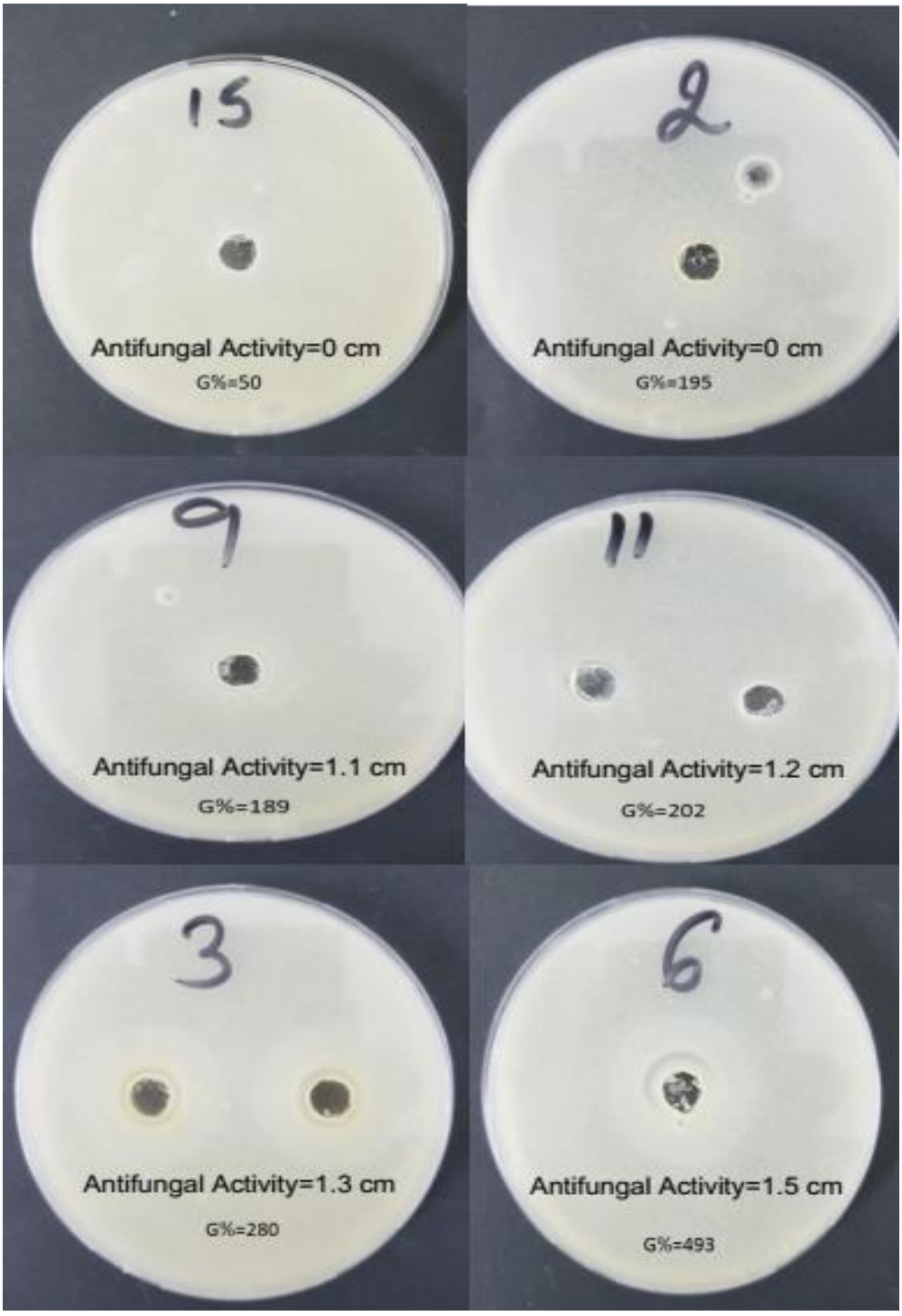

Figure 2.16. Antifungal analysis of pullulan-*graft*-NVI samples with grafting yields of (50%, 195%, 189%, 202%, 280%, 493%) against *Candida albicans* fungi

2.4 Conclusion

A neutral polysaccharide, pullulan, has been modified by grafting PNVI onto the polymer backbone in aqueous solution. The highest calculated grafting yield is G%=513. Pullulan-*graft*-PNVI copolymers obtained in this study are water soluble with potential biomedical applications such as acting as drug conjugate, gene carrier, or drug carrier. The graft copolymer synthesized gave complexes in aqueous acidic medium upon mixing with sodium tripolyphosphate and sodium citrate solutions. Hence, cationic nature has been imparted onto pullulan.

The modified grafted pullulan showed antifungal and antibacterial activities due to presence of imidazole ring and amine group on its backbone. Having antifungal and antibacterial properties make the novel copolymer usable in medicine industry.

Chapter 3

GRAFTING OF POLY(N-VINYLIMIDAZOLE) ONTO EPICHLOROHYDRIN CROSSLINKED PULLULAN HYDROGELS

Pullulan can form gels reactively with suitable crosslinking agents since it has nine hydroxyl groups available on the repeating unit [49]. Pullulan gels and their interaction with the enzyme lysozyme were studied to grasp the performance of immobilization, purification and separation of the enzymes and controlled release drug systems by crosslinking with sodium trimethaphosphate and epichlorohydrin. Crosslinked pullulan can be used to cure and heal infected wounds due to its action as an antibacterial agent and fluid adsorbent [50].

Hydrogel of pullulan was prepared in aqueous transparent solution by using sodium trimetaphosphate as the crosslinking agent. Some properties of this hydrogel such as being easily handled and cut to the desired thickness and shapes enable it to be used in vascular engineering [16]. Pullulan hydrogel was prepared with the purpose of using it for drug delivery for tumor extracellular in human body [17]. Crosslinking reaction of a carbohydrate polymer named pullulan by using a suitable crosslinking agent, epichlorohydrin, as an example is shown in Figure 3.1.

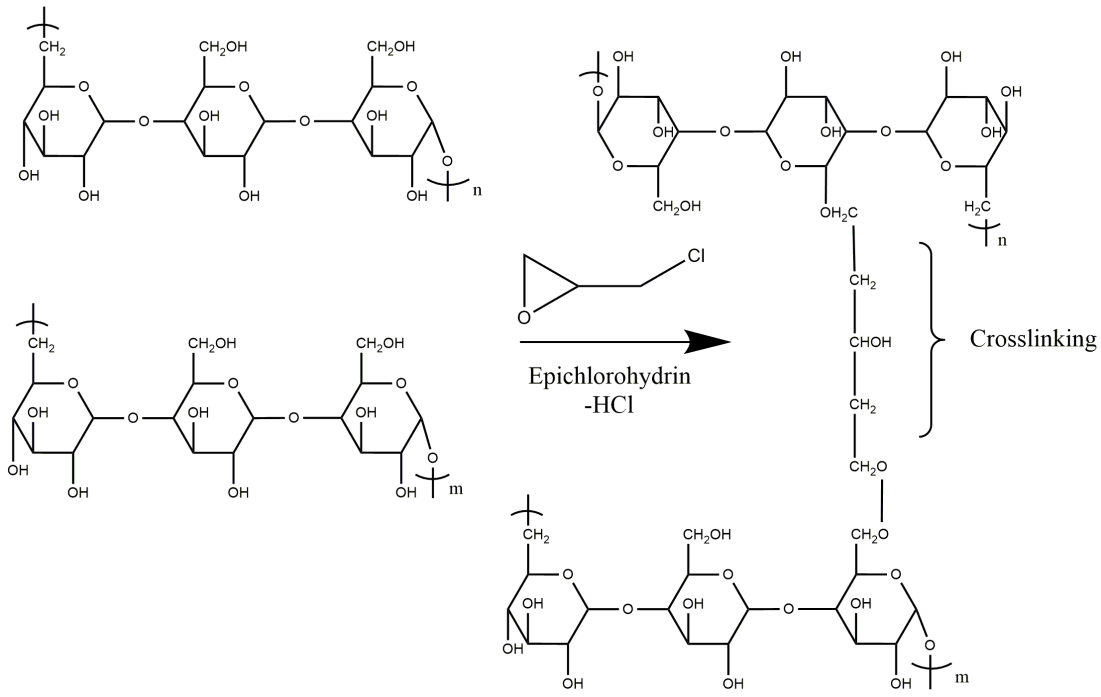

Figure 3.1. Chemically crosslinked pullulan structure by using epichlorohydrin

This part of the thesis describes synthesis and characterization of ECH crosslinked pullulan gel and grafting of PNVI onto the gel by free radical initiation under heterogeneous medium.

3.1 Experimental

3.1.1 Material

Pullulan (Hayashibara, Japan), N-vinyl imidazole (Aldrich, Germany), ammonium persulphate (Sigma Aldrich, Germany), sodium hydroxide (Sigma, Germany), epichlorohydrin (Sigma Aldrich, Germany), hydrochloric acid (Sigma, Germany), ethanol (SAFA, North Cyprus) were used without any further purification.

3.1.2 Pullulan Hydrogel Preparation (Pullulan-ECH)

To prepare crosslinked pullulan, 10 mL of a solution of 8.0 g/L pullulan and 2.5 M epichlorohydrin (ECH) dissolved in 10 mL of 5 M sodium hydroxide solution and reacted for 7 hours. The produced crosslinked pullulan was crashed with a mixer, then it was washed with distilled water and neutralized with 0.05 M HCL. Neutralization

was repeated for three times, then the product was treated with ethanol. After all, the product was dried at 40°C for 24 h under vacuum and pullulan gel was obtained. The product solubility in aqueous medium was checked. Obtained gel has high swelling property in aqueous and acidic solution.

3.1.3 Synthesis of Pullulan-ECH-*graft*-PNVI under Heterogeneous Conditions

Weighted amount of crosslinked pullulan (0.20 g) was allowed swell in 25 mL of 0.1 M of hydrochloric acid for 24 h. N-vinylimidazole (NVI) and the initiator, ammonium persulphate (APS), were added into the heterogeneous medium under nitrogen atmosphere and the reaction was carried out at constant temperature with continues stirring on a magnetic stirrer for a given period of time. The grafting system is shown in Figure 2.1. The preparation conditions are given in Table 3.1. The obtained product was filtered and washed with water and then with ethanol to remove any homopolymer formed and extract the extra water from the gel. Gels were dried at 40°C for 24 h under vacuum. Two experimental runs were carried out for each sample, and grafting yields were reported as the average of two samples with ±7-10% error. Grafting yields (G%) were calculated by using Equation (2.1), Table 3.1.

Table 3.1. Grafting conditions and grafting yields for pullulan-ECH-*graft*-PNVI

No	Pullulan-ECH (g/L)	[NVI] M	[APS] M	Time (h)	Temp (°C)	G%
1	8.0	0.09	0.18	3	40	25
2	8.0	0.18	0.18	3	40	23
3	8.0	0.35	0.18	3	40	55
4	8.0	0.44	0.18	3	40	96
5	8.0	0.57	0.18	3	40	189
6	8.0	0.66	0.18	3	40	189

3.2 Characterization Studies

FTIR-ATR analysis was carried out using Perkin Elmer Spectrum-Two in Eastern Mediterranean University, North Cyprus. Elemental analysis was carried out at Central Laboratory of METU in Ankara using LECO, CHNS-932 instrument. X-ray diffraction (XRD) analysis was carried out at Central Laboratory of METU in Ankara using Rigaku Ultima-IV X-Ray diffraction instrument. Thermal Gravimetric Analysis (TGA) analysis was carried out at Central Laboratory of METU using Perkin Elmer Pyris 1 instrument in Ankara. The samples were heated at a rate of 10°C/min under nitrogen atmosphere. The swellings tests were carried out in Eastern Mediterranean University, North Cyprus physical chemistry laboratory at room temperature.

3.3 Results and Discussion

3.3.1 Effect of Grafting Conditions on Grafting Yields under Heterogeneous Condition

Since the highest grafting yield under homogenous condition was obtained using 0.18 M ammonium persulphate and 8.00 g/L pullulan at 40°C and 3 h reaction duration showing in Table 2.1 [6], the grafting yield of N-vinylimidazole onto crosslinked pullulan under heterogeneous condition was optimized by changing in NVI monomer concentration (0.09, 0.18, 0.35, 0.44, 0.57 and 0.66 M), using constant initiator (0.18 M) and constant crosslinked pullulan gel (0.2 g dispersed in 25 mL acid solution) under 40°C and with 3h reaction duration, Table 3.1. It was observed that by increasing monomer amount, grafting yield increased to G%=189, after this level the grafting yield became constant upon further monomer increasing from 0.57 M to 0.66 M. After reaching a maximum value, increase in monomer amount does not cause further increase the grafting yield but leads to levelling off, Figure 3.2. A similar behavior was observed at grafting PNVI onto pullulan [6] and grafting PNVI onto chitosan [37]. The

tendency to level off with NVI concentration can be attributed to possible degradative chain transfer reactions, which hinder further polymerization of NVI. Also steric hindrance due to the grafted chains on pullulan backbone may be another factor hindering any further grafting [6,36,37,43].

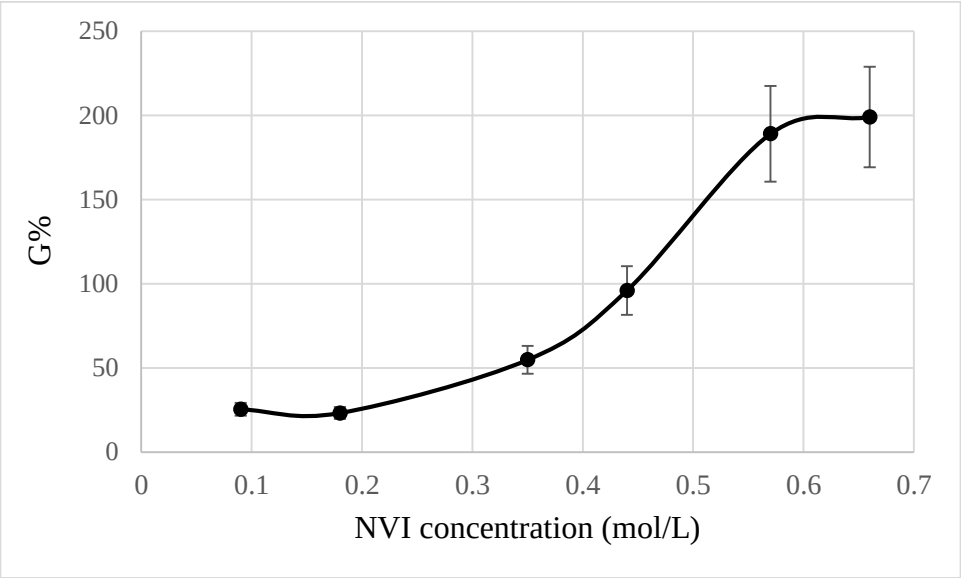

Figure 3.2. Effect of monomer concentration on grafting yield (G%) under heterogeneous condition

3.3.2 Elemental Analysis

For finding more evidence for PNVI grafting onto pullulan, elemental analysis was carried out on samples. H%, N% and C% values of the grafted gels obtained by increasing the monomer amount at 40°C for 3 h reaction duration using 0.2 g crosslinked pullulan in 25 mL of 0.1 M hydrochloric acid are reported in Table 3.2. It can be followed from the table that N% content of the obtained gels increase by increasing grafting yield value determined by gravimetric analysis, providing further evidence for imidazole grafting on the pullulan chain. The sample with low grafting yield (G%=23) has got the lowest nitrogen percentage (N%=1.81). The sample with the higher grafting yield (G%=189) bears the highest nitrogen percentage (N%=6.99).

Furthermore, G_E% values were calculated according to N% values obtained from elemental analysis, using Equation (2.3). The values G_E% show a similar trend as the G% values. It is important to have imidazole ring grafted on pullulan to provide the neutral polysaccharide with cationic property in aqueous acid medium. Having cationic functionality such as absorbability, antifungal and antimicrobial activity [6,35,37].

Table 3.2. Elemental analysis results of hydrogels (N%, C%, H%), gravimetric grafting yield and elemental analysis grafting yield

Sample	**Pullulan-ECH (g)**	**[NVI] M**	**C%**	**H%**	**N%**	**G%**	**G_E%**
Pullulan-ECH	-	-	39.53	6.15	-	-	-
1	0.20	0.18	36.81	6.03	1.81	23	17.5
2	0.20	0.35	37.05	5.98	2.55	55	27.9
3	0.20	0.57	37.79	6.12	6.99	189	133

3.3.3 FTIR-ATR Analysis

In the FTIR-ATR spectrum of crosslinked pullulan, at 3337 cm^{-1} the O-H stretching vibrations are observed. The C-H vibrations of the ECH crosslinked pullulan appear at 2929 cm^{-1}. The C-O and C-C stretching vibrations appear at 1644 cm^{-1}, 1148 cm^{-1}, 1078 cm^{-1}, 1009 cm^{-1}. The FTIR-ATR spectrum of PNVI homopolymer gives peaks at 2848 cm^{-1}, 2922 cm^{-1} and 3143 cm^{-1} are due to C-H stretching and N-H stretching vibrations respectively. The stretching vibration of C=N and C=C of the imidazole ring appear at 1641 cm^{-1} and 1556 cm^{-1}, respectively. The C-N vibrations are observed at 1490 cm^{-1} and 1300 cm^{-1} region [44]. In the spectra of the grafted products prepared under heterogeneous conditions having grafting yield of 189% characteristics of both crosslinked pullulan and PNVI can be observed in Figure 3.3. In addition to C-H vibrations due to crosslinking observed at 2888 cm^{-1}, glycosidic and etheric bonds of

pullulan was observed in the region of 1250-900 cm^{-1}, characteristic C=N stretching bonds of PNVI were observed at 1644-1575-1522 cm^{-1} region and C-N stretching bond of PNVI were observed at 1258 cm^{-1} region in the grafted sample spectra. The O-H stretching of the grafted sample is less intense than that of the pullulan gel. This feature provides evidence for grafting reaction occurring on the –OH sites in pullulan.

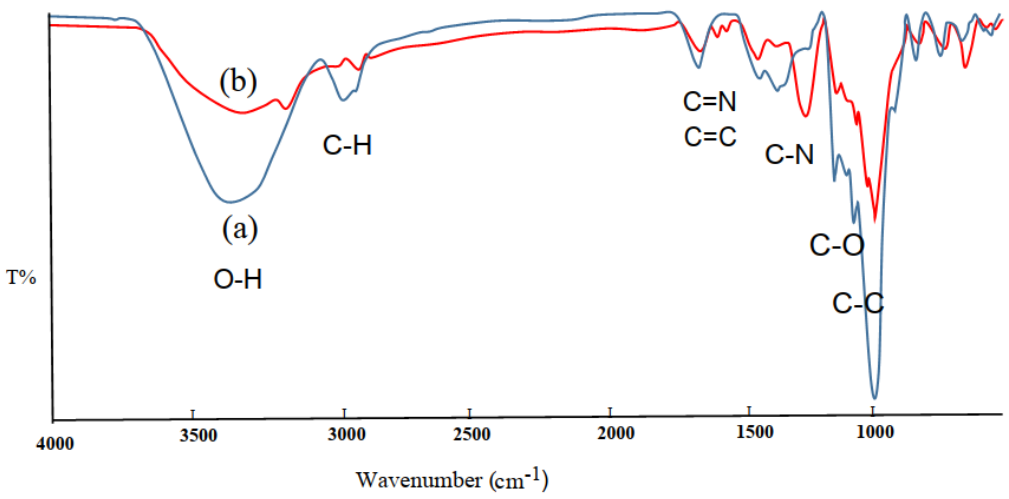

Figure 3.3. FTIR-ATR spectrum of (a) pullulan-ECH and (b) pullulan-ECH-*graft*-PNVI hydrogels

3.3.4 Swelling Test

Swelling property of the prepared hydrogels was tested at room temperature in aqueous medium. 0.05 g of each sample was weighted and swollen either in distilled water or 0.1 M hydrochloric acid. Weight of the swollen samples were recorded at each 10 minutes for duration of 24 h. Swelling percentage was calculated by using Equation (3.1). The equilibrium state of hydrogel both swollen in distilled water and 0.1 M hydrochloric acid is shown in Figure 3. (4 and 5) respectively.

$$S\% = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100\% \quad (3.1)$$

Swelling percentage capacities both in distilled water and 0.1 M hydrochloric acid show that increasing monomer concentration brings about lower swelling percentage due to increasing H-bonding interactions between amine groups and hydroxyl groups that exist on the copolymer backbone. Increasing number of amine groups on copolymer backbone increase the probability of H-bonding formation. As long as H-bonding formation increased absorbability of the hydrogel decreased.

Equilibrium state of distilled water absorption for pullulan-ECH and pullulan-ECH-*graft*-PNVI hydrogels with 189%, 54% and 23% grafting yield was determined as 1100%, 700%, 1200% and 1300% respectively at 25°C in distilled water. By increasing monomer amount, grafting percentage increases and swelling percentage decreases. Pullulan-ECH hydrogel exhibits higher swelling capacity than pullulan-ECH-*graft*-PNVI (G%=189). This observation can be explained by increased H-bonding at higher grafting values, Figure 3.4.

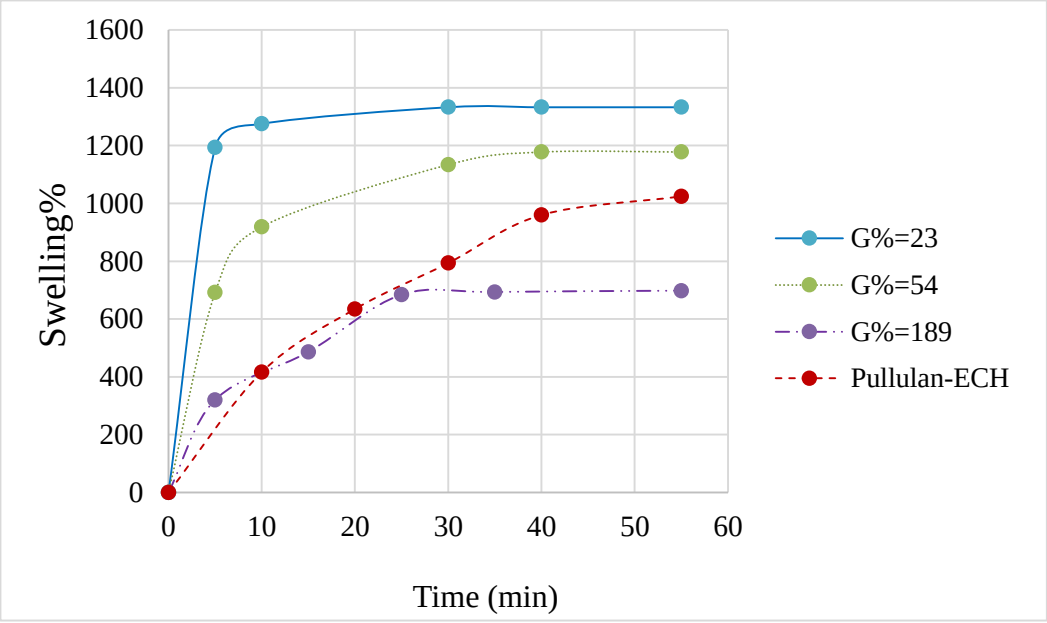

Figure 3.4. Water absorption capacity of pullulan-ECH and pullulan-ECH-*graft*-PNVI (G%=23, G%=54 and G%=189) hydrogels in distilled water at room temperature

Swelling percentage of the grafted hydrogels boosts up enormously due to amine groups protonation in acidic medium solution in compare to its swelling percentage in water solution. Equilibrium state of pullulan-ECH and pullulan-ECH-*graft*-PNVI with 189%, 54% and 23% grafting yield was determined as 1100%, 6000%, 7000% and 8500%, respectively at 25°C in 0.1 M hydrochloric acid, Figure 3.5. PNVI grafted samples have much higher swelling capability in acid solution than the pullulan-ECH sample due to the presence of the basic imidazole group in the structure. However, similar to the swelling behaviour in distilled water, equilibrium swelling values decrease with increasing grafting yield. This behaviour indicates a tighter network structure with increasing grafting yield that may be due to more H-bonding interaction with grafted NVI moieties and alcohol functionalities on pullulan with increasing grafting yield. Swelling percentage result shows that pullulan-ECH-*graft*-PNVI copolymers are able to absorb a large amount of water. Due their absorbability property we can classify them as super absorbent hydrogels. Superabsorbent hydrogels are polymeric materials with ability of absorbing or retaining huge amounts of water or other bio-fluids in aqueous or biological environment [51].

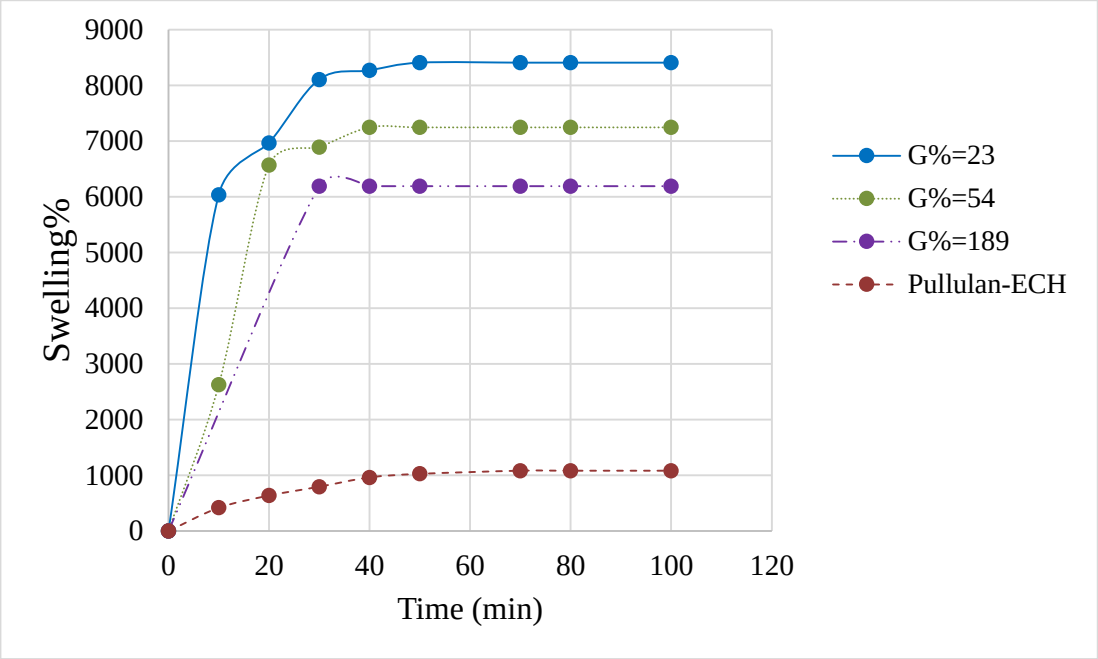

Figure 3.5. Water absorption capacity of pullulan-ECH and pullulan-ECH-*graft*-PNVI (G%=23, G%=54 and G%=189) hydrogels in 0.1 M hydrochloric acid at room temperature

3.3.5 TGA Analysis

TGA thermogram of pullulan, Pullulan-ECH and pullulan-ECH-*graft*-PNVI (G%=189), is given in Figure 3.6. Pullulan loses water up to 130°C. There is a 10% weight loss due to water loss. Similarly, pullulan-ECH and Pullulan-ECH-*graft*-PNVI lose water in 25°C-160°C range with 10% weight loss. While pullulan decomposes sharply at 325°C to 20% of its original weight, the crosslinked sample decomposes at the same temperature range but at a slower rate than pullulan [6]. This behaviour shows that crosslinked pullulan has higher thermal stability than non crosslinked pullulan. crosslinked pullulan thermal stability is more than pullulan.

Two stages of weight loss is obvious at TGA curve of pullulan-ECH-*graft*-PNVI. Grafted sample started to decompose at a lower temperature than crosslinked pullulan (210°C) with 30% weight loss. Then decomposition continues slower at higher temperature (330°C) compare to pullulan and crosslinked pullulan decomposition

temperature. Having two distinct decomposition regions prove the presence of both pullulan and PNVI in the grafted sample structure. After decomposition 24% of the crosslinked pullulan sample and 32% of the grafted sample remain at 600°C. Upon further heating pullulan completely decomposes at 600°C. Hence, grafted sample has higher thermal stability than crosslinked pullulan and pullulan samples respectively. This observation supports the idea given above in section 3.3.4 that a tighter network is formed at higher grafting yields.

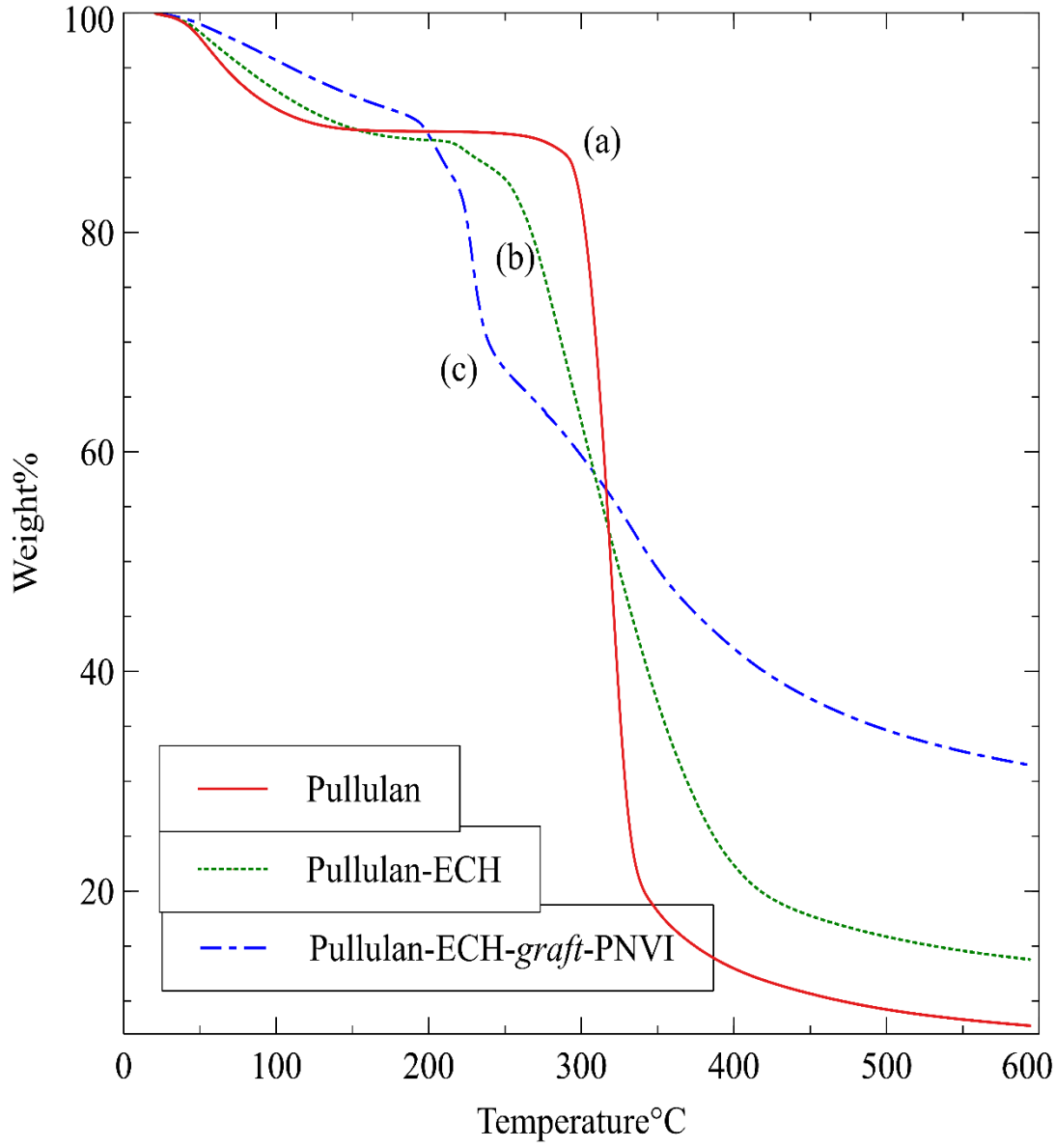

Figure 3.6. TGA curve of (a) pullulan, (b) pullulan-ECH and (c) pullulan-ECH-*graft*-PNVI

3.3.6 X-Ray Diffraction

X-Ray diffraction patterns of pullulan, crosslinked pullulan (pullulan-ECH) and PNVI grafted onto crosslinked pullulan (pullulan-ECH-*graft*-PNVI) are shown in Figure 3.7. A broad peak at 2Θ=18° appears due to the semicrystalline nature of the polysaccharide together with an amorphous hump at 2Θ=36° in both pullulan and pullulan-ECH samples. The peaks shift to 2Θ=21° and the amorphous hump shifts to 2Θ=45° in pullulan-ECH-*graft*-PNVI. The relative intensity of the peak at 2Θ=21° decreases indicating that the amorphous nature of the sample is more enhanced in the grafted pullulan gel compared to the nongrafted samples [26,54].

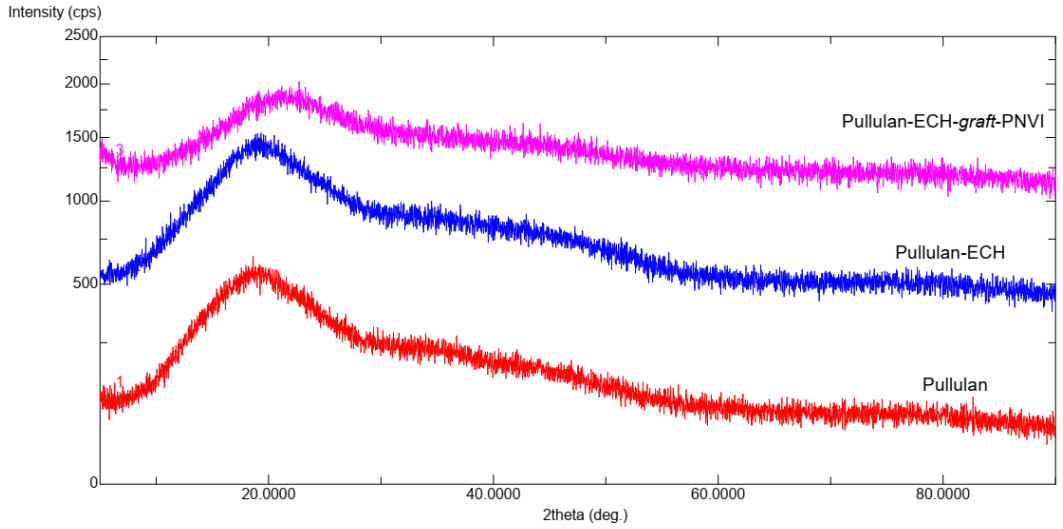

Figure 3.7. X-Ray diffraction of pullulan, pullulan-ECH and pullulan-ECH-*graft*-NVI

3.4 Conclusion

Epichlorohydrin crosslinked pullulan hydrogel proved to be suitable materials for PNVI grafting in aqueous acidic solution. The highest calculated grafting yield was found as 189%. The pullulan-ECH-*graft*-PNVI copolymers are superabsorbent hydrogels with equilibrium swelling capacities of 700% to 1300% in distilled water in room temperature and 6000% to 8500% in acidic solution in room temperature.

Therefore they act as pH responsive hydrogels. Pullulan-ECH-*graft*-PNVI copolymers obtained in this study are cationic superabsorbent hydrogels available for biomedical and environmental applications under suitable conditions.

Chapter 4

PULLULAN/POLY(N-VINYLIMIDAZOLE) CRYOGELS

Cryogels are hydrogels with 3D hydrophilic polymer networks formed by chemical crosslinking or hydrogen bonds. They are prepared under a freezing condition via a freeze-thaw cycle in aqueous medium. Polymeric cryogels are formed upon freezing solutions or colloidal dispersions of polymers at moderately low temperatures, allowing a frozen stage for a given period of time and then thawing of the system. The method produces porous polymeric gels due to the existence of a multiphase system in the frozen state. Both frozen solvent molecules, i.e. ice crystals and a small fraction of nonfrozen solution containing dissolved species (the unfrozen liquid microphase) coexist in the frozen state. The gel formation reaction occurs in the nonfrozen liquid microphase, and the ice crystals act as porogen leading to the porous morphology of the gel [55]. Several precursor systems such as colloidal dispersions, solutions of monomeric or polymeric precursors, solutions of self gelling polymers, or solutions of polyelectrolytes containing crosslinking ionic agents offer cryogel formation possibilities [55]. The polymer network that forms under cryogelation conditions contain interconnected large pores since the solvent acts as porogen during the gel formation [26]. Crosslinking occurs around ice crystals in the frozen medium. The macropores that form are highly interconnected, and exhibit sponge-like morphology. They bear mechanical and chemical stability [56]. They act as hydrogels as they contain hydrophilic side groups, in general. Due to being a simple and cheap

preparation method in comparison to other kinds of hydrogels, cryogelation is receiving increasing interest [20].

The porous nature, chemical and mechanical stability and hydrophilicity of cryogels allow them to have various applications [56] such as microbiology applications, biomedical applications [20,21,22,24,26,56], tissue engineering [23,26], drug delivery [57] and heavy metal removal [58]. They are attractive separation and purification matrices as well. This makes them suitable for isolation of large biomolecules, as well as for chromatographic separation of cells, organelles, virus particles and environmental applications for bioremediation [56]. Cryogel applications in the microbiology field are also available for processing cell and virus suspensions and cell culture applications [24].

Poly(vinyl alcohol) cryogels are one of the most widely studied examples in this field. For biomedical applications, poly(vinyl alcohol) cryogels and poly(vinyl alcohol) composites with cellulose and chitosan were prepared separately by using freeze-thaw cycling process [26]. Poly(vinyl alcohol) cryogels and its composites are suitable materials for medical device applications such as orthopedic devices, intervertebral discs, cartilage and cardiovascular devices, including vascular grafts and heart valves [26]. Another application named for poly(vinyl alcohol) cryogels and its composite is tissue engineering application since their mechanical properties closely match the tissue and is compatible with the tissue environment [26,54].

There are studies on natural based polysaccharides derivatives cryogels [23] and their composites [57]. Dextran methacrylate and hyaluronan methacrylate are polysaccharide based cryogels prepared under homogenous condition by freeze

cycling method. Preliminary cytotoxicity tests illustrated excellent cytocompatibility of the cryogels making them especially attractive as matrices in tissue regeneration procedures [23]. A new cryogel for controlled release of enalapril maleate drug was prepared based on two biodegradable polymers named pullulan and poly(vinyl alcohol) as the matrix of polymer and zeolite L as an inorganic filler of the product [57].

A competitive adsorption study on poly(2-hydroxyethyl methacrylate-N-vinyl imidazole) cryogel and poly(2-hydroxyethyl methacrylate-N-vinyl imidazole)/poly(2-hydroxyethyl methacrylate) composite cryogel were performed in the removal of Pb^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+} ions from aqueous solutions. Cryogels could be used for ten repetitive adsorption and desorption processes without significant loss in the adsorption capacity [58].

There is another study on imidazole based cryogels modified by various alkyl bromides. Poly(N-vinylimidazole) cryogels were prepared as simultaneous polymerization via cryopolymerization technique. The crosslinking occur around ice crystals in the frozen medium of the system. Poly(N-vinylimidazole) cryogels impart positive charge to the cryogels. They were modified with 1,2-dibromoethane, 1,4-dibromobutane and 1,6-dibromohexane. Poly(N-vinylimidazole) cryogels are found as multipurpose materials, in terms of chemical modification and in situ metal nanoparticle preparation, providing superior control of H_2 production via externally applied magnetic field, and usefulness as active, fast, reusable catalyst system for H_2 generation reactions from hydrolysis of $NaBH_4$ [59].

In this part of the thesis, effect of preparation conditions on the properties of Pullulan-ECH, PNVI, PNVI-ECH, and Pullulan-PNVI-ECH cryogels has been investigated. The cryogels were prepared under different reactions by changing the pullulan and NVI concentrations, ECH concentration, time and temperature.

4.1 Experimental

4.1.1 Material

Pullulan (Hayashibara, Japan), N-vinyl imidazole (Aldrich, Germany), ammonium persulphate (Sigma Aldrich, Germany), sodium hydroxide (Sigma, Germany), epichlorohydrin (Sigma Aldrich, Germany), ethanol (SAFA, North Cyprus) were used without any further purification.

4.1.2 Pullulan, PNVI and Pullulan/PNVI Cryogels Preparation

To prepare pullulan cryogel, pullulan was dissolved in distilled water to prepare 40% (w/v), 20% (w/v) and 10% (w/v) pullulan solutions. Then 0.2 mL of 2% (w/v) sodium hydroxide solution was added to 0.5 mL of each pullulan solution. After cooling down the solutions for 3 h at 4°C, 0.05 mL, 0.5 mL and 1.0 mL epichlorohydrin (ECH) was added into the solution under mild magnetic stirring.

To prepare PNVI cryogel, NVI was dissolved in distilled water to prepare 4% (v/v), 30% (v/v) and 60% (v/v) monomer solutions. After cooling down the solutions for 3 h, 0.5 g, 1.0 g and 2.0 g APS was added into 6.5 mL of monomer solution at 4°C under mild magnet stirring.

To prepare pullulan/PNVI cryogel, prepared pullulan solution and monomer solution were mixed under magnet stirring to prepare homogenous solutions.

All prepared solutions were frozen in the freezer for 24 h, 48 h and 72 h under -18°C, -22°C and -24°C temperature. The prepared gels were taken out and washed by distilled water and ethanol to remove unreacted initiator, monomer or any other unreacted molecule. Ethanol was used to extract water molecules from the gels. Prepared gels were freeze dried. Two experimental runs were carried out for each sample, gel fraction percentage were calculated as the average of two samples with ±10% error. Gel fraction percentage (GF%) were calculated by using Equation (4.1) and given in Table 4.1.

$$GF\% = \frac{W_d}{W_0} \times 100\% \quad (4.1)$$

Where;

W_d=weight of dried cryogel after swelling in distilled water for 24 h (g), W_0=initial weight of dried cryogel (g).

PNVI percentage content (PNVI%) of cryogels were calculated using N% content reported by elemental analysis according to the following calculation:

$$NVI(g) = N(g) \times \frac{N\ (mol)}{N(g)} \times \frac{NVI\ (mol)}{2N\ (mol)} \times \frac{NVI\ (g)}{NVI\ (mol)}$$

This can be summarized into the following formula, Equation (4.2).

$$PNVI\% = \frac{N*MW_2}{2*MW_1} \times 100\% \quad (4.2)$$

Where;

N=nitrogen amount in 100 grams of the cryogel reported by elemental analysis (g), MW_1=molecular weight of nitrogen=14.01 (g/mol), MW_2=molecular weight of monomer, NVI=94.1145 (g/mol).

Yield percentage of cryogels were calculated using Equation (4.3).

$$Y\% = \frac{W}{W_1+W_2+W_3} \times 100\% \quad (4.3)$$

Where;

W=weight of dried cryogel (g), W_1=initial weight of pullulan (g), W_2= initial weight of NVI (g), W_3=initial weight of ECH (g).

4.2 Characterization Studies

FTIR-ATR analysis was carried out using Perkin Elmer Spectrum-Two spectrometer in Eastern Mediterranean University, North Cyprus. The following analysis were carried out central laboratory of METU in Ankara. Elemental analysis was carried out using LECO, CHNS-932 instrument. X-ray diffraction (XRD) analysis was made using Rigaku Ultima-IV X-Ray diffraction instrument. Thermal gravimetric analysis (TGA) was carried out using Perkin Elmer Pyris 1 instrument. The samples were heated at a rate of 10°C/min under nitrogen atmosphere. Scaning electoron microscopy (SEM) analysis was carried out at Central Laboratory of METU using QUANTA 400F Field Emission instrument in Ankara. The swelling tests of the samples were carried out in Eastern Mediterranean University, North Cyprus physical chemistry laboratory at room temperature.

4.3 Results and Discussion

The cryogels of pullulan are formed due to the crosslinking reaction between pullulan and ECH together with H-bonding interaction between pullulan chains. Crosslinking reaction between pullulan and ECH is a well established one, and has been reported at higher temperatures before [50]. The crosslinking reaction between pullulan and ECH is shown Figure 3.1. A schematic representation of pullulan cryogel is given in Figure 4.1.

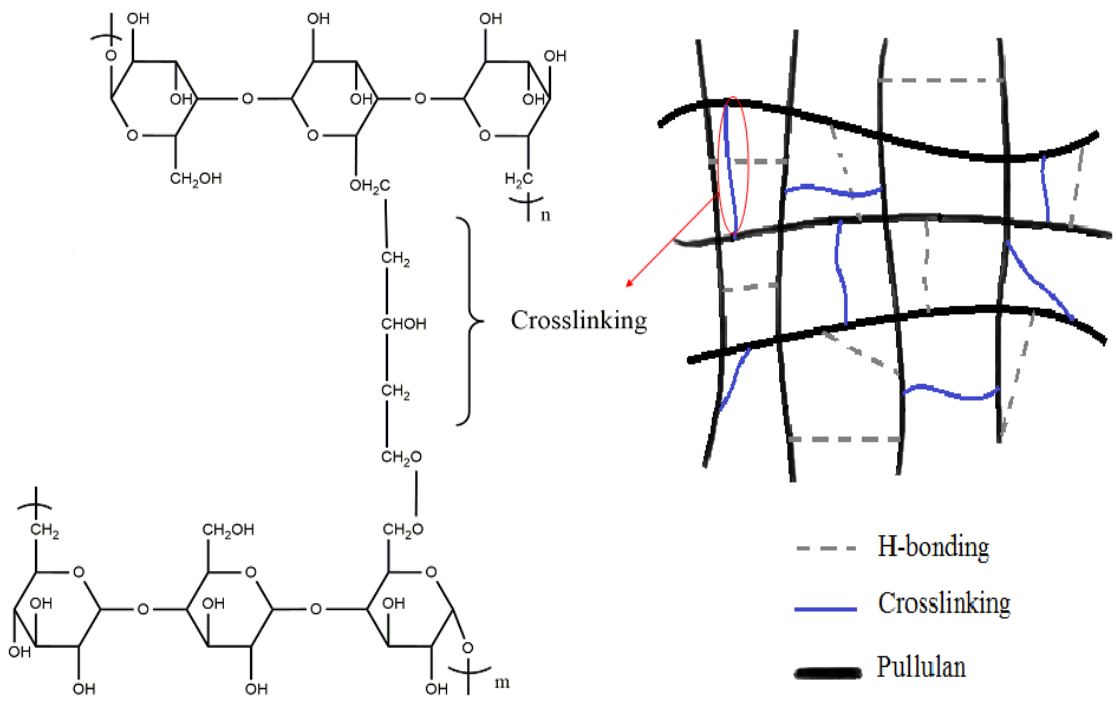

Figure 4.1. A schematic representation of pullulan cryogel

The crosslinking reaction of N-vinylimidazole monomer using with epichlorohydrin as a crosslinking agent is shown in Figure 4.2.

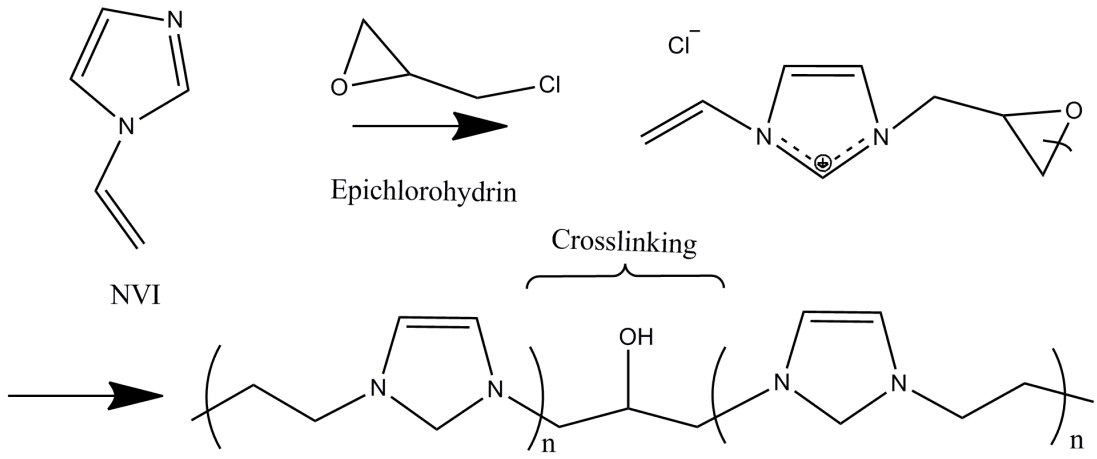

Figure 4.2. Chemically crosslinked N-vinylimidazole structure by using epichlorohydrin

PNVI form cryogels in the absence of the chemical crosslinker, ECH, as well. PNVI polymer networks should be stabilized by strong dipole-dipole interactions and by H-bonding interaction due to trapped water molecules acting as bridges among PNVI chains. Elemental analysis results indicate presence of water molecules within PNVI cryogel structure. A schematic representation is given in Figure 4.3.

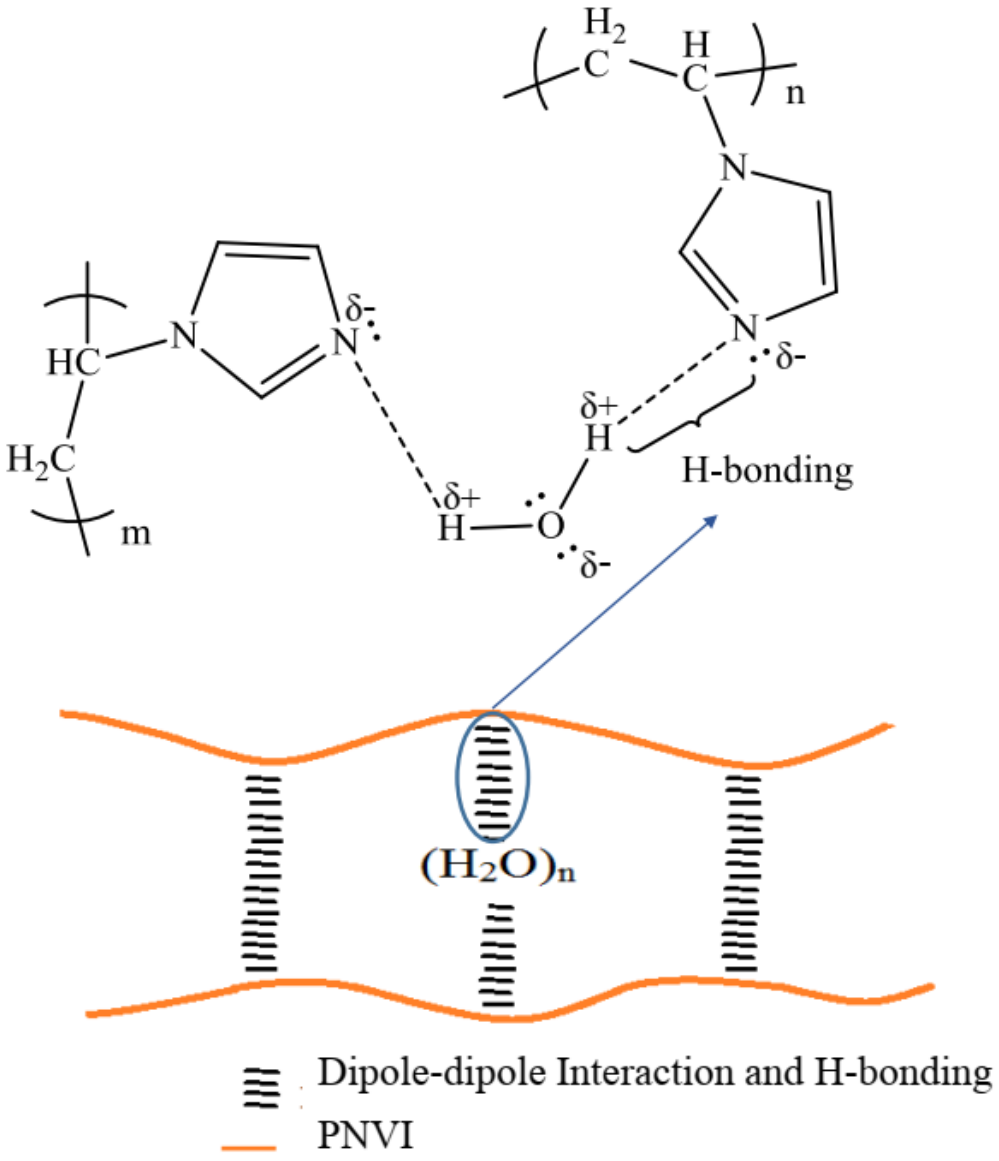

Figure 4.3. A schematic representation PNVI cryogel structure

Pullulan/PNVI cryogels are made up of pullulan-ECH and PNVI-ECH crosslinked structures and epichlorohydrin crosslinked and PNVI grafted pullulan chains. These structures may interact via dipole-dipole and H-bonding interactions, as proposed in Figure 4.4.

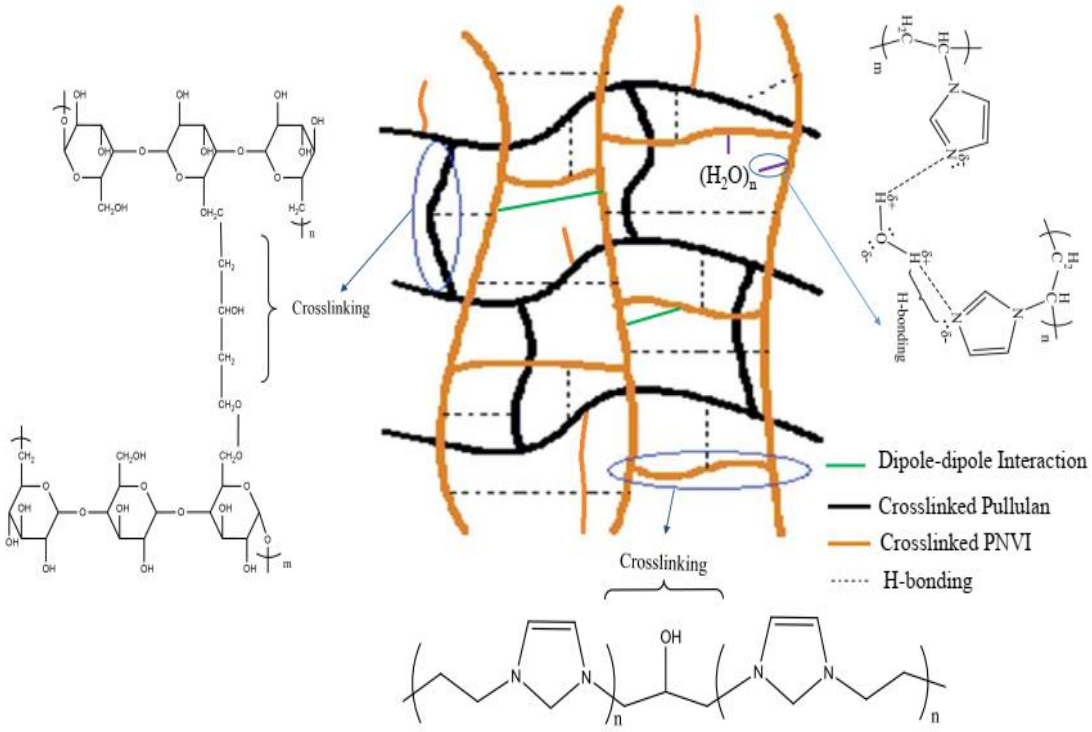

Figure 4.4. Schematic structure of pullulan/PNVI gel formation

4.3.1 Effect of Reaction Conditions on Cryogels Gel Fraction Percentage

Pullulan, PNVI and pullulan/PNVI cryogels were prepared under conditions given in Table 4.1. Crosslinking reaction of pullulan and a schematic representation of pullulan gelation structure are given in Figure 3.1, Figure 4.1 respectively. Crosslinking reaction of NVI monomer and a schematic representation of PNVI gelation structure are given in Figure 4.2 and Figure 4.3 respectively. A schematic representation of pullulan/PNVI gel formation is given in Figure 4.4.

Gel fraction percentages of cryogels were optimized according to the effect of monomer concentration (0.44 M, 3.31 M and 6.62 M), pullulan concentration (10 (g/L), 20 (g/L), 40 (g/L), 100 (g/L), and 200 (g/L)), initiator concentration (0.38 M, 0.76 M and 1.52 M), crosslinking agent concentration (0.70 M, 7.00 M and 14.0 M), gel formation duration (24 h, 48 h and 72 h) and temperature (-18°C, -22°C and -24°C) of the reaction medium. Gel fraction percentages of the gels were calculated by using Equation (4.1). Optical pictures of a set of the prepared cryogels are shown in Figure 4.5. Gel formation reaction conditions and gel fraction percentages of cryogels are reported in Table 4.1.

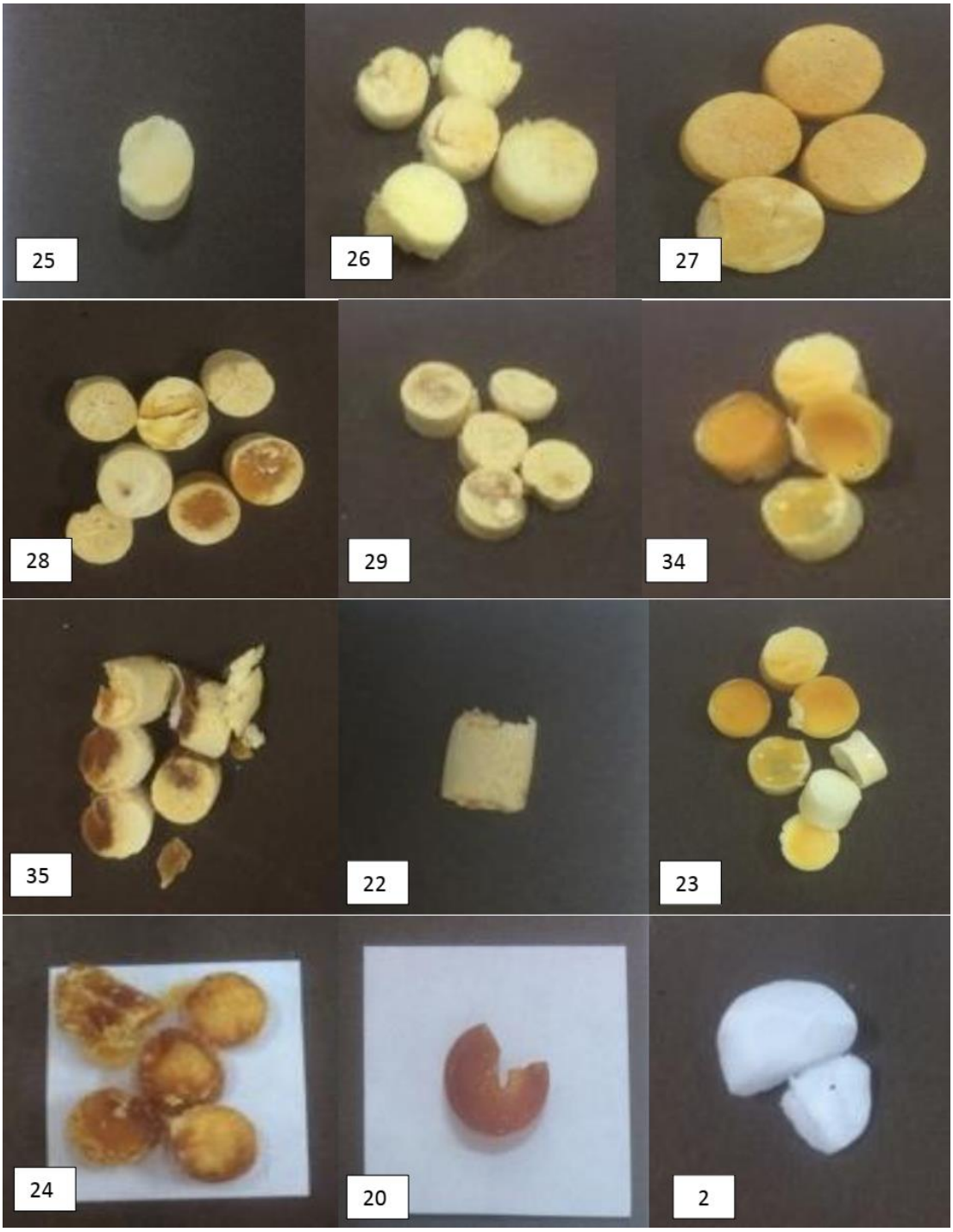

Figure 4.5. Digital picture of a set of cryogel samples prepared under different reaction conditions reported in Table 4.1

Table 4.1. Gel formation conditions, yield percentage and gel fraction percentage (GF%) of cryogels

No	[Pullulan] (g/L)	[NVI] M	[APS] M	[ECH] M	Temp	Time (h)	Y%	GF%	Observation
1	40.0	-	-	0.70	-18	48	64	20	Gel
2	40.0	-	-	7.00	-18	48	32	96	Gel
3	40.0	-	-	14.0	-18	48	19	96	Gel
4	20.0	-	-	7.00	-18	48	20	97	Gel
5	10.0	-	-	7.00	-18	48	19	99	Gel
6	40.0	-	-	7.00	-22	48	57	90	Gel
7	40.0	-	-	7.00	-24	48	55	70	Gel
8	40.0	-	-	7.00	-18	24	52	93	Gel
9	40.0	-	-	7.00	-18	72	24	98	Gel
10	-	6.62	0.76	7.00	-18	48	100	98	Gel
11	-	3.31	-	7.00	-18	48	-	-	No Gel
12	-	3.31	0.38	-	-18	48	80	60	Gel
13	-	3.31	0.76	-	-18	48	100	67	Gel
14	-	3.31	1.52	-	-18	48	44	62	Gel
15	-	3.31	0.76	-	-22	48	63	67	Gel
16	-	3.31	0.76	-	-24	48	57	66	Gel
17	-	3.31	0.76	-	-18	24	100	56	Gel
18	-	3.31	0.76	-	-18	72	60	97	Gel
19	-	3.31	0.76	0.70	-18	48	82	93	Gel
20	-	3.31	0.76	7.00	-18	48	90	95	Gel
21	-	3.31	0.76	14.0	-18	48	80	96	Gel
22	40.0	0.44	0.76	0.70	-18	48	34	9.0	Gel
23	40.0	3.31	0.76	0.70	-18	48	49	59	Gel
24	40.0	6.62	0.76	0.70	-18	48	97	87	Gel
25	40.0	0.44	0.76	7.00	-18	48	40	12	Gel
26	40.0	3.31	0.76	7.00	-18	48	47	85	Gel
27	40.0	6.62	0.76	7.00	-18	48	94	88	Gel
28	100	6.62	0.76	7.00	-18	48	100	97	Gel
29	200	6.62	0.76	7.00	-18	48	72	97	Gel
30	40.0	6.62	0.38	7.00	-18	48	69	82	Gel
31	40.0	6.62	1.52	7.00	-18	48	70	83	Gel
32	40.0	6.62	0.76	7.00	-18	24	62	80	Gel
33	40.0	6.62	0.76	7.00	-18	72	73	89	Gel
34	40.0	6.62	0.76	7.00	-22	48	84	87	Gel
35	40.0	6.62	0.76	7.00	-24	48	85	86	Gel
36	40.0	6.62	0.76	14.00	-18	48	97	89	Gel
37	-	0.44	0.76	7.00	-18	48	78	42	Gel

4.3.1.1 Pullulan Cryogels Gel Fraction Percentage and Yield Percentage Optimization

To optimize the gel fraction of pullulan cryogels, different cryogel samples were prepared under different reaction conditions, by changing pullulan and ECH concentrations under different temperatures and at different freezing time as given in Table 4.1. The maximum gel fraction, GF%=99, is found for sample 5 at -18°C for 48 h duration using 10 (g/L), pullulan and 7.00 M ECH. However, this sample gives the lowest yield by 19%.

4.3.1.1.1 Effect of Pullulan Concentration on Gel Fraction Percentage and Yield Percentage of Pullulan Cryogel

Effect of pullulan concentration on pullulan cryogels gel fraction percentage was investigated by changing pullulan concentration (10 (g/L), 20 (g/L), and 40 g/L) under constant reaction condition, 7.00 M ECH under -18°C for 48 h duration, Samples 5, 4 and 2. According to Figure 4.6, increasing amount of pullulan results in higher yield. Furthermore, gel fractions of the products are the same. Increasing pullulan concentration, does not affect the gel fraction. The reason for increasing Y% with increasing pullulan concentration should be the contribution of H-bonding interactions in between pullulan chains in addition to chemical crosslinking with ECH. Increasing pullulan concentration brings pullulan chains together in the unfrozen microphases under cryogelation conditions allowing increased H-bonding interactions. The gel that is isolated after thawing of the cryogelation system consists of chemically crosslinked and H-bonded pullulan chains. Gel fractions, which are obtained after water treatment of the gel increase only slightly with pullulan concentration indicating that the products obtained were crosslinked network structures with only a small (1-4%) soluble fraction.

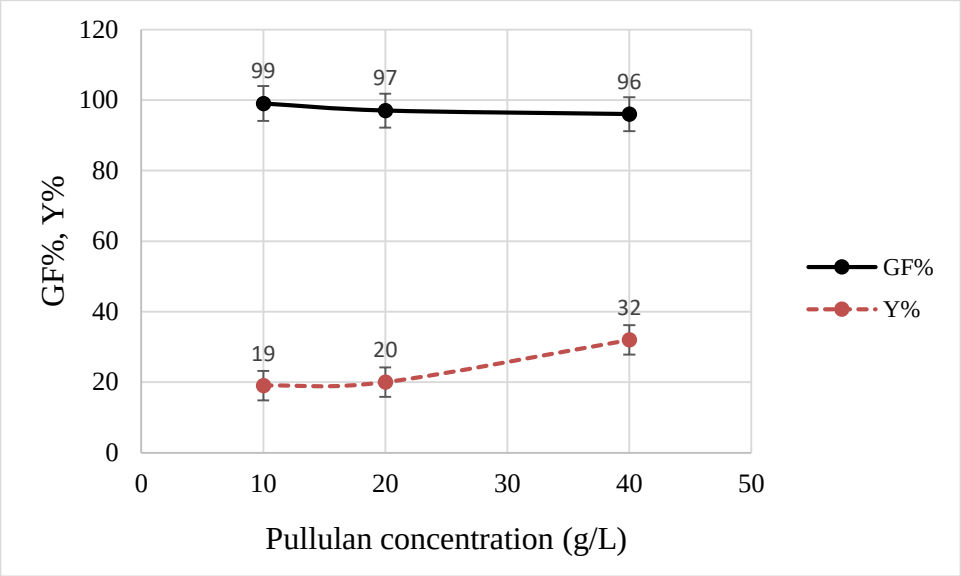

Figure 4.6. Effect of pullulan concentration on pullulan cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 7.00 M ECH under -18°C for 48h reaction duration

4.3.1.1.2 Effect of ECH Concentration on Pullulan Cryogel Gel Fraction Percentage and Yield Percentage of Pullulan Cryogel

Effect of ECH concentration on pullulan cryogels gel fraction percentage was investigated by changing ECH concentration (0.7 M, 0.07 M and 14.0 M) under constant reaction condition, -18°C for 48 h reaction duration, Samples 1, 2 and 3. According to Figure 4.7, gel fraction percentage increases by increasing crosslinking concentration from 20% to 96%. Yield percentage decreases by increasing ECH amount because not all of the ECH used initially, is inserted into the gel structure. But, GF% is 96% at 7.00 M ECH, therefore that concentration in taken as the optimum concentration for ECH. By more detailed analysis, it can be followed from Figure 4.6 that at 0.7 M ECH concentration, Y% is the highest with 64% but GF% is the lowest with 20%. When ECH concentration is increased to 7.0 M and further to 14.0 M, the yield decreases to 32% and 19% respectively. However gel fractions of the products increase drastically to 96%. An explanation for this behavior may be attempted by considering the chances for ECH crosslinking and H-bonding interactions between

pullulan chains. At low ECH concentrations, H-bonding interactions contribute to gel formation to a greater extent increasing the yield. Some uncrosslinked pullulan chains might be trapped within the network structure, which increase the yield. After water treatment, the H-bonding may be disturbed and some of the network structure might be lost. Uncrosslinked and non H-bonded chains dissolve away decreasing the GF%. At higher ECH concentrations, crosslinking reaction becomes more probable, giving rise to almost fully crosslinked pullulan cryogels. The yields show a decreasing trend at higher ECH concentrations, since the contribution of H-bonding to network formation decreases. There should be ECH crosslinked pullulans rather than H-bonded pullulans in the network structure. The yield, however, does not increase with increasing ECH concentration, because pullulan is the limiting factor. There should be excess ECH in the medium.

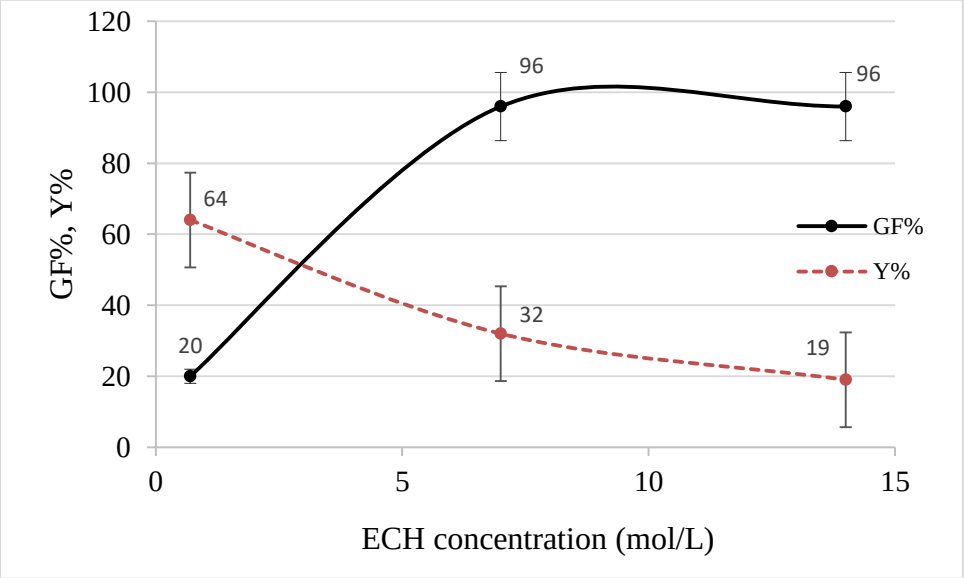

Figure 4.7. Effect of ECH concentration on pullulan cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 40 (g/L) pullulan under -18°C for 48h reaction duration

4.3.1.1.3 Effect of Temperature on Gel Fraction Percentage and Yield Percentage of Pullulan Cryogel

The effect of temperature was evaluated by decreasing temperature of the gelation reaction from -18°C to -24°C, Samples 2, 6 and 7. By decreasing the temperature from -18°C to -24°C gel fraction increases from 70% to 96%. Yield percentage decreases from 55% to 32% by increasing the temperature. The highest gel fraction percentage, GF%= 96, is found out at -18°C, Figure 4.8. As the freezing environment freezing environment becomes colder, increasing weight of obtained gel brings about higher yield percentage, however gel fraction percentage decreased. At lower temperatures, the probability of H-bonding interaction inbetween pullulan chains within the nonfrozen microphases increases leading to higher yields. However, only ECH crosslinked chains remain after water treatment as some of the H-bonded pullulan chains might dissolve away together with free pullulan chains trapped within the cryogel network structure. Therefore, the GF% decreases with decreasing temperature.

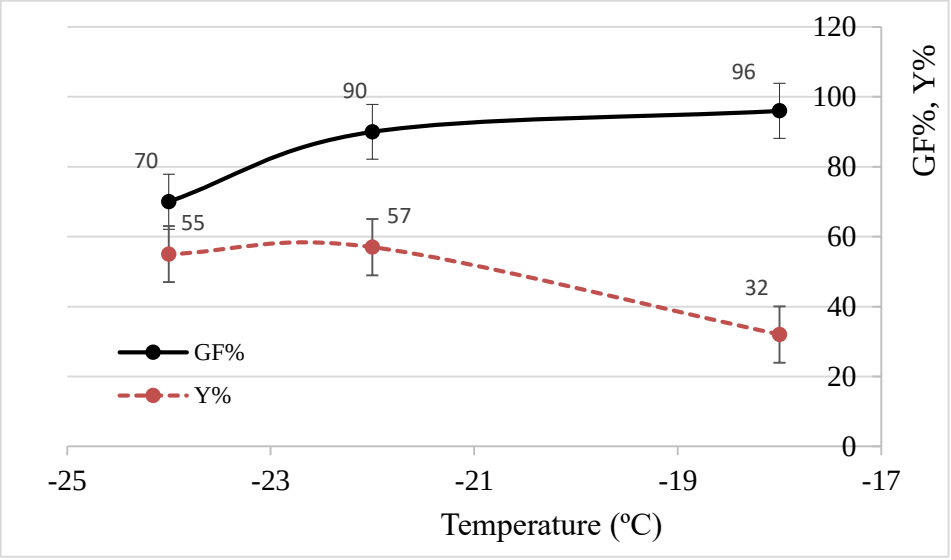

Figure 4.8. Effect of temperature on pullulan cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 40 (g/L) pullulan, 7.00 M ECH under 48h reaction duration

4.3.1.1.4 Effect of Time on Gel Fraction Percentage and Yield Percentage of Pullulan Cryogel

According to Figure 4.9, Samples 8, 2 and 9, increasing reaction duration under any other constant reaction condition, causes no significant change in gel fraction percentage. The gel fraction values changing from 93% to 98% in 24 h to 72 h of freezing duration. Yield percentage decreased from 52% to 24% by increasing reaction duration from 24 h to 72 h as weight of prepared gel for all samples using constant pullulan and ECH concentration under different reaction duration were constant. In time, some of the free pullulan chains trapped within the network structure may diffuse out of the cryogel formed leading to lower yield percentages but relatively higher GF percentages.

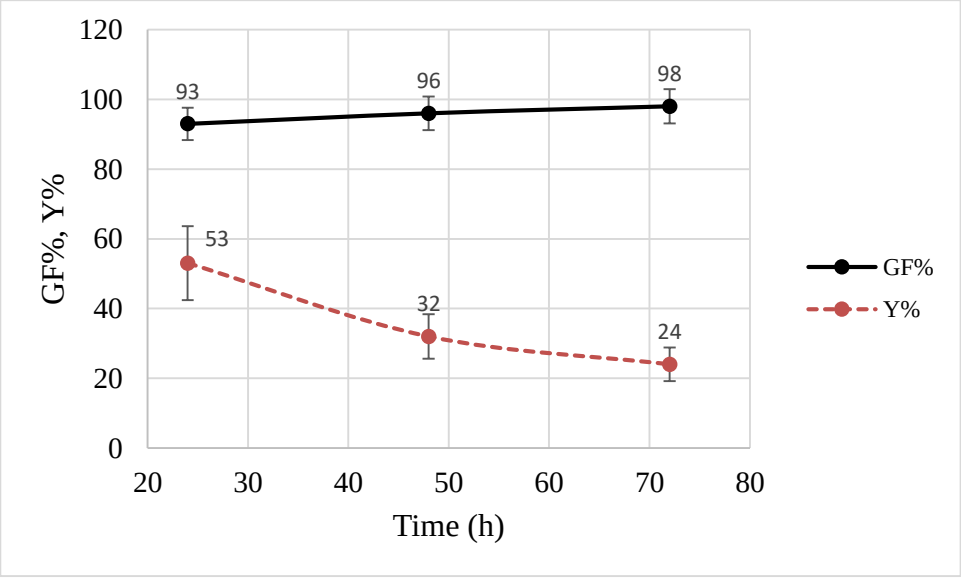

Figure 4.9. Effect of time on pullulan cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 40 (g/L) pullulan, 7.00 M ECH under -18°C

4.3.1.2 PNVI Cryogels Gel Fraction Percentage and Yield Percentage Optimization

PNVI cryogel formation reaction occurs using APS as an initiator. APS creates active sites on monomer structure and initiate gelation reaction. H-bonding formation may happen through the H-bonding interaction between nitrogen atoms in amine groups of NVI ring and trapped water molecules within the network structure. PNVI should interact via dipole-dipole interactions as well. PNVI cryogel formation was also investigated using ECH in the reaction medium. Crosslinking reaction occurs between nitrogen atoms of NVI ring and the crosslinking agent. Crosslinking reaction structure between NVI monomer and ECH and the schematic representation of the PNVI gel are given in Figure 4.2 and Figure 4.3, respectively. Gel fraction of PNVI cryogels was optimized by changing monomer concentration, APS concentration, ECH concentration, reaction temperature and gelation duration. Sample 38 reported in Table 4.1 shows the maximum gel fraction, GF%=98, is found at -18°C, for 48 h duration using 6.62 M NVI, 7.00 M ECH and 0.76 M APS.

4.3.1.2.1 Effect of Monomer Concentration on Gel Fraction Percentage and Yield Percentage of PNVI Cryogels

NVI concentration effect on PNVI cryogels was studied by changing NVI concentration (0.44 M, 3.31 M and 6.62 M) under constant reaction condition, samples 37, 20 and 10 are reported in Table 4.1. As it follows from Figure 4.10, gel fraction percentages found as 42%, 95% and 98% respectively. By increasing monomer concentration amount gel fraction percentage increased from 42% to 98%. The highest gel fraction percentage, GF%=98, is found at -18°C, for 48 h reaction duration using 6.62 M NVI, 7.00 M ECH and 0.76 M APS. Yield percentage increased by increasing NVI concentration under any other constant reaction condition. The reason of

increasing yield percentage and gel fraction percentage by increasing monomer concentration under constant reaction condition may be H-bonding interaction formation between amine group on imidazole ring with any hydrogen available in the medium from water or the dipole-dipole interactions between the imidazole groups themselves, Figure 4.2.

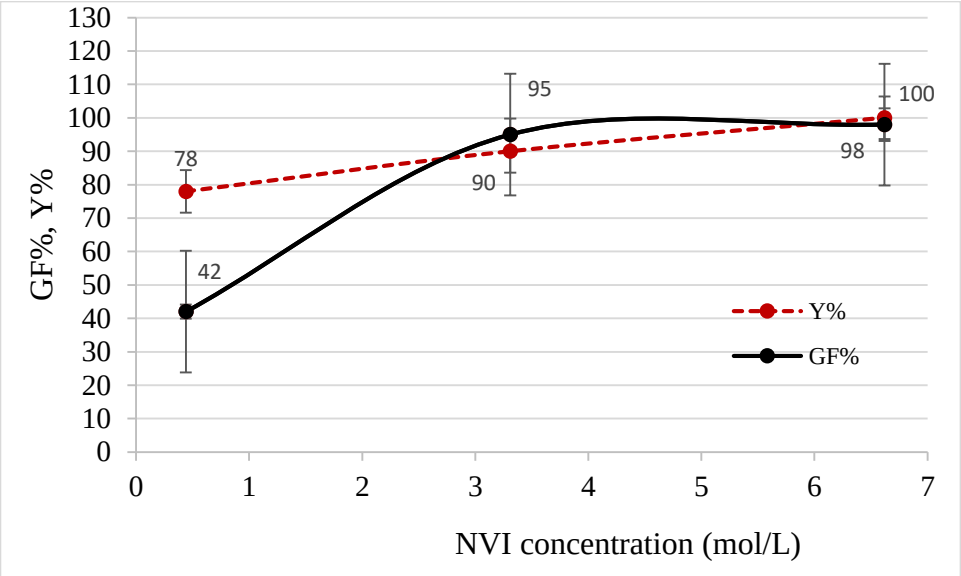

Figure 4.10. Effect of monomer concentration on PNVI cryogels gel fraction (GF%) and yield percentage (Y%) using 7.00 M ECH, 0.76 M APS under -18°C for 48h reaction duration

4.3.1.2.2 Effect of ECH Concentration on Gel Fraction Percentage and Yield Percentage of PNVI Cryogels

Effect of ECH concentration on gel fraction percentage of PNVI cryogels was investigated by changing ECH concentration (0.00 M, 0.70 M, 0.07 M and 14.0 M) under constant reaction condition, (Samples 13, 19, 20 and 21). Gel fraction percentage increased by increasing amount of crosslinking agent concentration from 67% to 96%. Figure 4.11. Yield percentage behavior shows that the optimum concentration of ECH for NVI gel formation is 7.00 M under constant reaction conditions. Gel formation of PNVI cryogel occurs under -18°C for 48 h duration without ECH usage in the reaction

medium. NVI monomer goes under gel formation reaction initiated by APS. Dipole-dipole interaction formation may occur between PNVI by itself or with hydrogen atoms in the medium, (Sample 13).

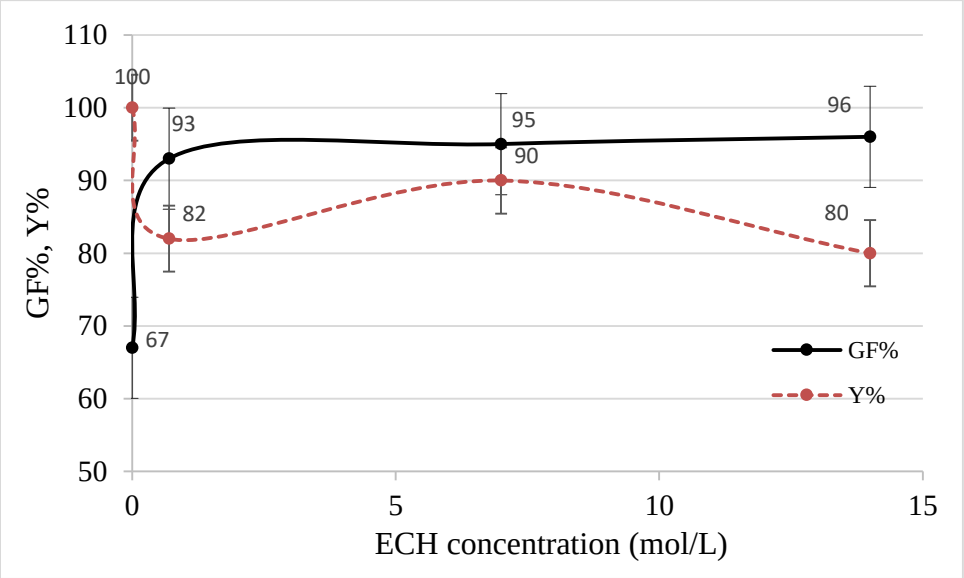

Figure 4.11. Effect of ECH concentration on PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 3.31 M NVI, 0.76 M APS under -18°C for 48h reaction duration

4.3.1.2.3 Effect of Initiator Concentration on Gel Fraction Percentage and Yield Percentage of PNVI Cryogels

In order to study the effect of initiator concentration on gel fraction of cryogels, various initiator concentration (0.38 M, 0.76 M and 1.52 M) were used by keeping any other variable constant, Samples 12, 13 and 14. Increasing initiator concentration does not have significant effect on gel fraction percentage since gel fraction amounts are changing in the range of 60%-67%. Increasing initiator concentration leads to increase in gel formation reaction of NVI monomer under freezing condition from 80% yield to a maximum value of 100% yield by using 0.76 M of APS, and then gel formation value passes off to 44% of yield as can be followed from Figure 4.12. Excess amount of APS in the medium, higher than 0.76 M, may prevent the gelation reaction.

Possibility of reacting persulphate radicals between each other is high when APS concentration is high in the medium. The highest weight gain of the gel is achieved using 0.76 M APS in the medium since yield percentage is 100%. It shows all monomer take place in gel formation reaction using 0.76 M APS under constant condition but since GF% is 67% some part of the gel is soluble in water.

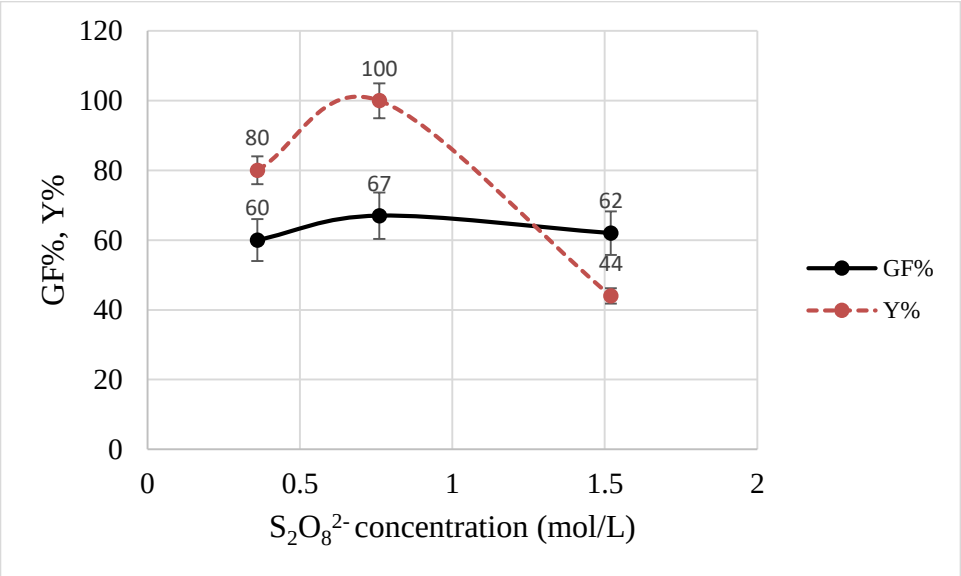

Figure 4.12. Effect of APS concentration on PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 3.31 M NVI under -18°C for 48h reaction duration

4.3.1.2.4 Effect of Temperature on Gel Fraction Percentage and Yield Percentage of PNVI Cryogels

Effect of temperature was evaluated by decreasing temperature of the gelation reaction as -18°C, -22°C and -24°C, under constant reaction condition, (Samples 13, 15 and 16). All samples are showing almost the same gel fraction percentage around 67, Figure 4.13. Since water molecules take part in cryogel formation, the probability of gel formation is not affected much with temperature. The highest yield percentage achieved at -18°C for 48 h reaction duration. The same probability of gel formation at -18°C, -22°C and -24°C can be considered as a factor contributing to almost same gel

fraction percentage. This should be due to higher amount of nonfrozen water molecules, which take part in cryogel formation at higher temperatures.

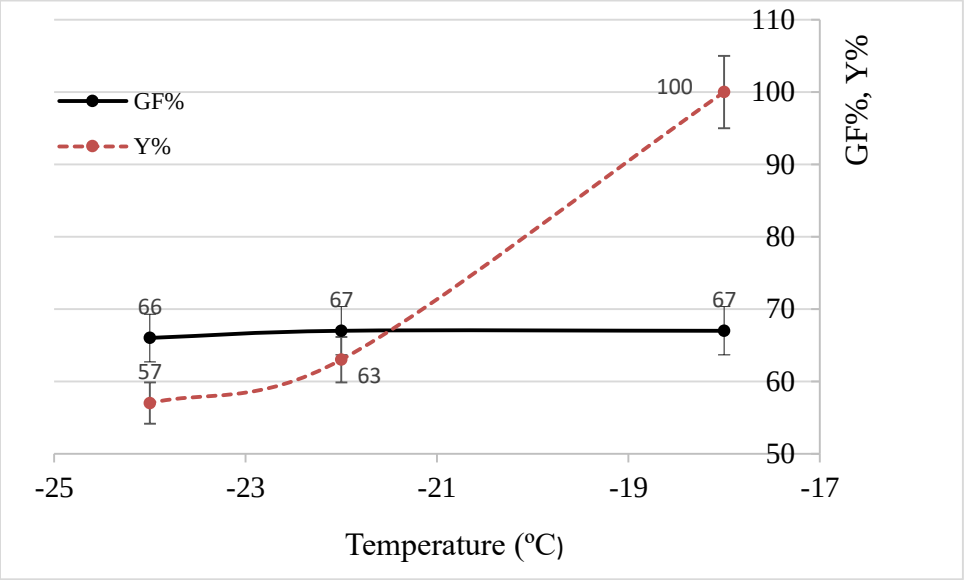

Figure 4.13. Effect of temperature on PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 3.31 M NVI, 0.76 M APS for 48h reaction duration

4.3.1.2.5 Effect of Time on Gel Fraction Percentage and Yield Percentage of PNVI Cryogel

To optimize the gel fraction values of PNVI cryogels and evaluate freezing duration effect on fraction yield, reaction duration is changed as 24 h, 48 h and 72 h under constant any other reaction condition, (Samples 17, 13 and 18). According to Figure 4.14, by increasing reaction duration under freezing condition from 24 h to 72 h, gel fraction percentage increased from 56% to 97%. Since Y% of the gel prepared in 72 h duration under constant condition decreased from 100% to 60%, but has highest gel fraction percentage 97% shows that weight of the gel is less than other samples but the gel is completely insoluble gel. In time, chain termination reactions and radical death may occur to a more probable extent and radical death may occur to a more probable extent. The yield of PNVI and hence crosslinked PNVI decreases.

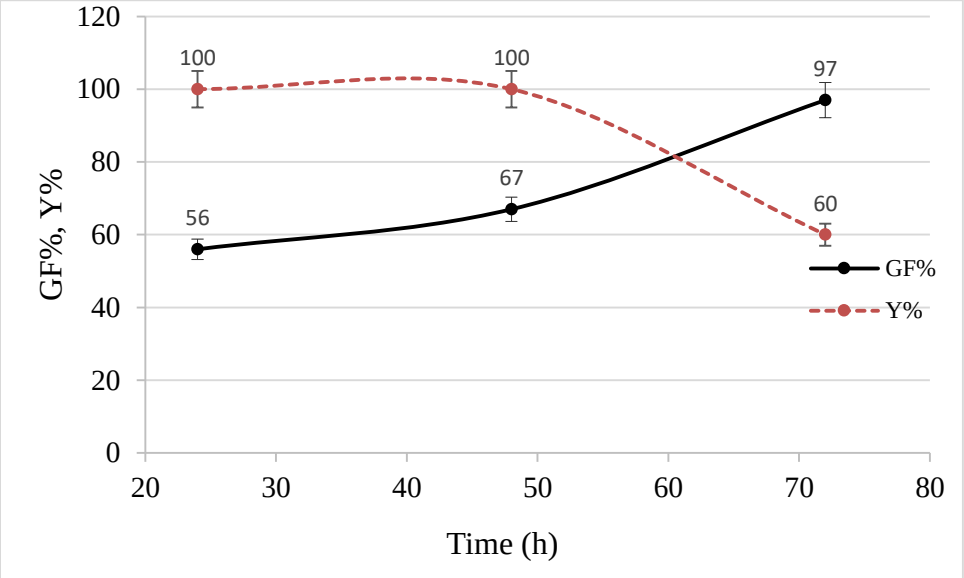

Figure 4.14. Effect of time on PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 3.31 M NVI, 0.76 M APS under -18°C

4.3.1.3 Pullulan/PNVI Cryogels Gel Fraction Percentage and Yield Percentage Optimization

Gel fraction of pullulan/PNVI cryogels were optimized by changing pullulan concentration, monomer concentration, APS concentration, ECH concentration, reaction temperature and gelation duration. The maximum gel fraction, GF%=97, is found at -18°C, for 48 h reaction duration using 100 (g/L) and 200 (g/L) pullulan, 6.62 M NVI, 7.00 M ECH and 0.76 M APS, (Samples 28 and 29). PNVI/pullulan cryogel samples are reported in Table 4.1.

4.3.1.3.1 Effect of Monomer Concentration on Gel Fraction Percentage and Yield Percentage of Pullulan/PNVI Cryogel

To study monomer concentration effect on gel fraction percentage and yield percentage, Sample 25, 26 and 27, were prepared using different monomer concentration (0.44 M, 3.31 M and 6.62 M) under other constant reaction condition, Figure 4.15. By increasing monomer concentration, gel fraction increased from 12% to 88% and yield percentage increased from 40% to 94%. Increased of gel fraction

and yield percentage by increasing NVI under constant reaction conditions can explain higher possibility of H-bonding and dipole-dipole interactions in between copolymer chain. Moreover, higher grafting yields are possible with more monomer available in the medium.

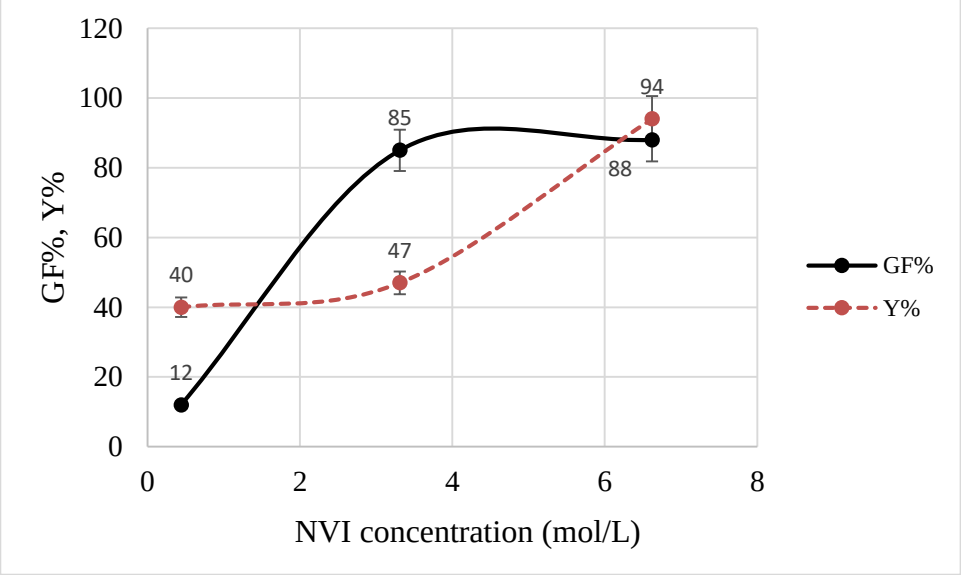

Figure 4.15. Effect of monomer concentration on pullulan/PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 40 (g/L) pullulan, 0.76 M APS and 7.00 M ECH under -18°C for 48h reaction duration

4.3.1.3.2 Effect of Pullulan Concentration on Gel Fraction Percentage and Yield Percentage of Pullulan/PNVI Cryogel

By increasing pullulan concentration as 40 (g/L), 100 (g/L) and 200 (g/L), Samples 27, 28 and 29, gel fraction percentage increases from 88% to 97% and reaches a maximum value of 97% when the amount of pullulan is 100 (g/L) as shown in Figure 4.16. A higher amount of pullulan provides higher number of possible sites available for gel formation and PNVI grafting. However, the number of crosslinking and grafting sites that can be created is limited by the amount of crosslinking agent and the amount of the monomer, which are factors limiting the gel fraction after 100 (g/L) pullulan concentration. Lowest yield percentage amount 72%, for sample with the

highest gel fraction percentage 97% shows excess amount of pullulan in the reaction medium under the same condition may bother gelation reaction formation. Higher amounts of pullulan after 100 (g/L) concentration results in same gel formation of the cryogel.

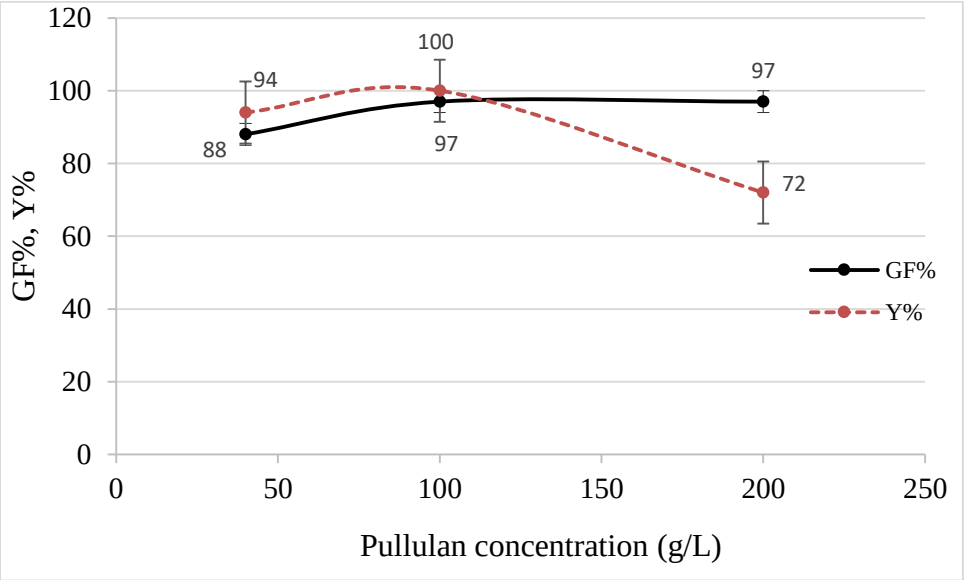

Figure 4.16. Effect of pullulan concentration on pullulan/PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 6.62 M NVI, 0.76 M APS and 7.00 M ECH under -18°C for 48h reaction duration

4.3.1.3.3 Effect of ECH Concentration on Gel Fraction Percentage and Yield Percentage of Pullulan/PNVI Cryogel

Effect of ECH concentration on Pullulan/PNVI cryogels gel fraction percentage and yield percentage was investigated by changing ECH concentration (0.70 M, 0.07 M and 14.0 M) under constant reaction condition, (Samples 24, 27 and 36), Figure 4.17. In gel formation of pullulan/PNVI cryogels crosslinking via dipole-dipole interaction and H-bonding may take place in between amine groups, hydrogens and -OH groups in the medium. This can be a reason of no significant change in gel fractions (87%, 88% and 89%) and yield percentage (97%, 94% and 97%) of samples by increasing

ECH amount under constant reaction condition. Since there is no active side available in the medium to form gel by crosslinking, excess amount of crosslinker may remain in the medium as the liquid phase of the gelation reaction.

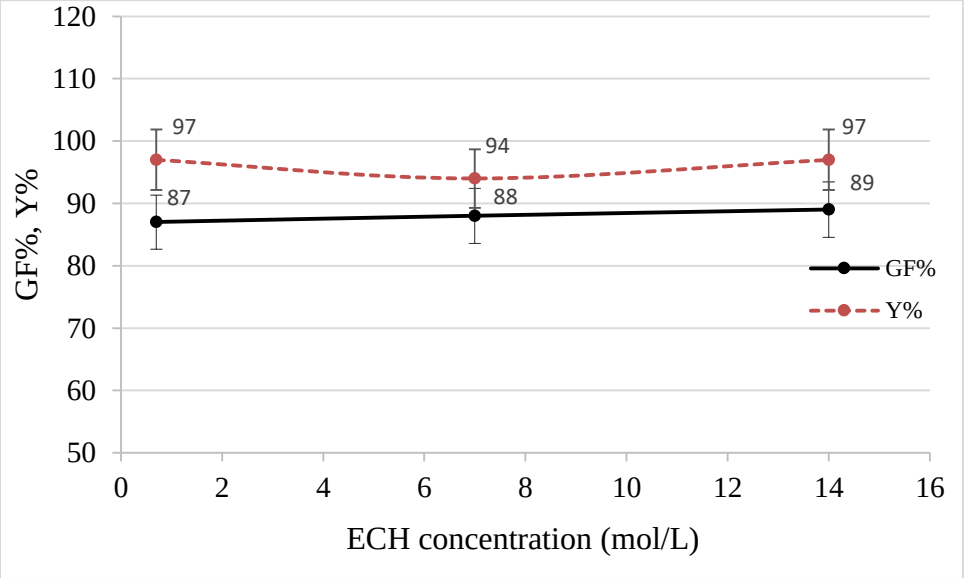

Figure 4.17. Effect of ECH concentration on pullulan/PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 6.62 M NVI, 0.76 M APS and 40 (g/L) pullulan under -18°C for 48h reaction duration

4.3.1.3.4 Effect of Initiator Concentration on Gel Fraction Percentage and Yield Percentage of Pullulan/PNVI Cryogel

To evaluate the effect of initiator concentration on gel fraction percentage, (Samples 30, 27 and 31), different amount of initiator concentration (0.36 M, 0.76 M and 1.52 M) were used by keeping any other variable constant. Increasing initiator concentration increased yield percentage from 69% to a maximum value of 94% by increasing APS concentration from 0.36 M to 0.76 M, and further increase in APS concentration to 1.52 M caused a decreased on the yield percentage value to 70%, as can be followed from Figure 4.18. Excess APS in the medium may bother the gelation reaction. Active initiator radicals may react together. High and almost the same value

of gel fraction percentages (82%, 88% and 83%) show that initiator concentration has no significant effect on the gels water solubility.

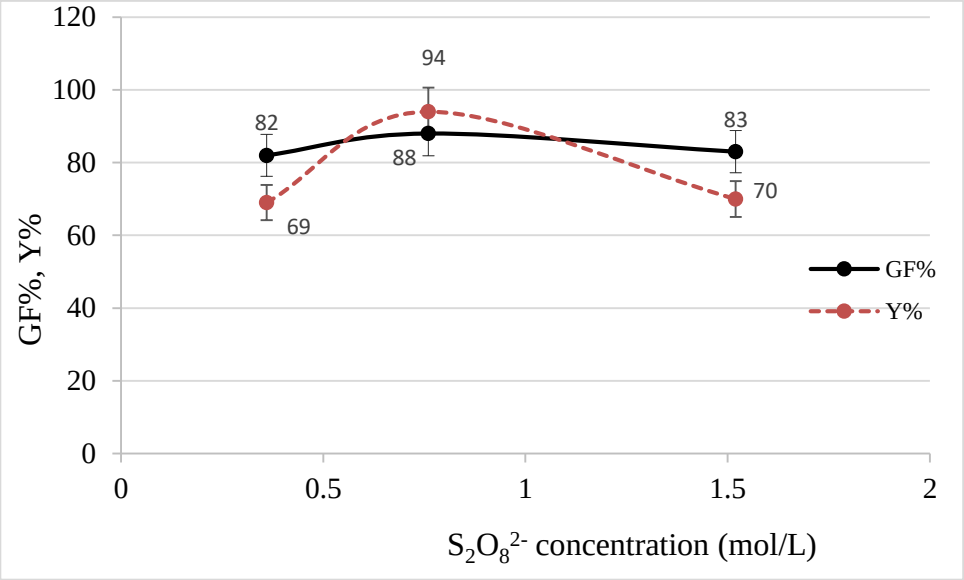

Figure 4.18. Effect of APS concentration on pullulan/PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 6.62 M NVI, 7.00 M ECH and 40 (g/L) pullulan under -18°C for 48h reaction duration

4.3.1.3.5 Effect of Temperature on Gel Fraction Percentage and Yield Percentage of Pullulan/PNVI Cryogel

Effect of Temperature was evaluated by decreasing temperature of the gelation reaction from -18°C to -24°C, (Samples 27, 34 and 35). By increasing temperature from -24°C to -18°C under constant reaction conditions, yield percentage increased from 85% to 94%, since the gel fraction percentage of samples are almost same such as (86%, 87% and 88%). The sample with highest yield percentage 94% has good gel fraction percentage 88%, found out under -18°C, Figure 4.19.

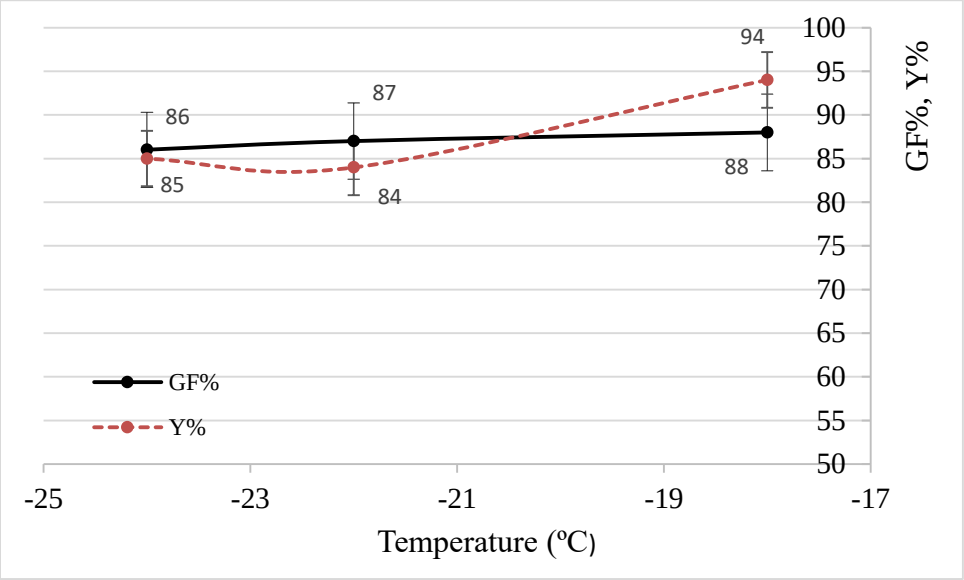

Figure 4.19. Effect of temperature on pullulan/PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 6.62 M NVI, 0.76 M APS, 7.00 M ECH and 40 (g/L) pullulan for 48h reaction duration

4.3.1.3.6 Effect of Time on Gel Fraction Percentage and Yield Percentage of Pullulan/PNVI Cryogel

To optimize the gel fraction and yield percentage of PNVI cryogels, reaction duration is changed from 24 h to 72 h under constant reaction condition, (Samples 32, 27 and 33). According to Figure 4.20, by increasing reaction duration under freezing condition from 24 h to 48 h, yield percentage increased from 62% to 94%. After 48 h of reaction duration, yield percentage decreased to 73%. Gel fraction percentage of samples increased slowly by time increasing from 80% to 89%.

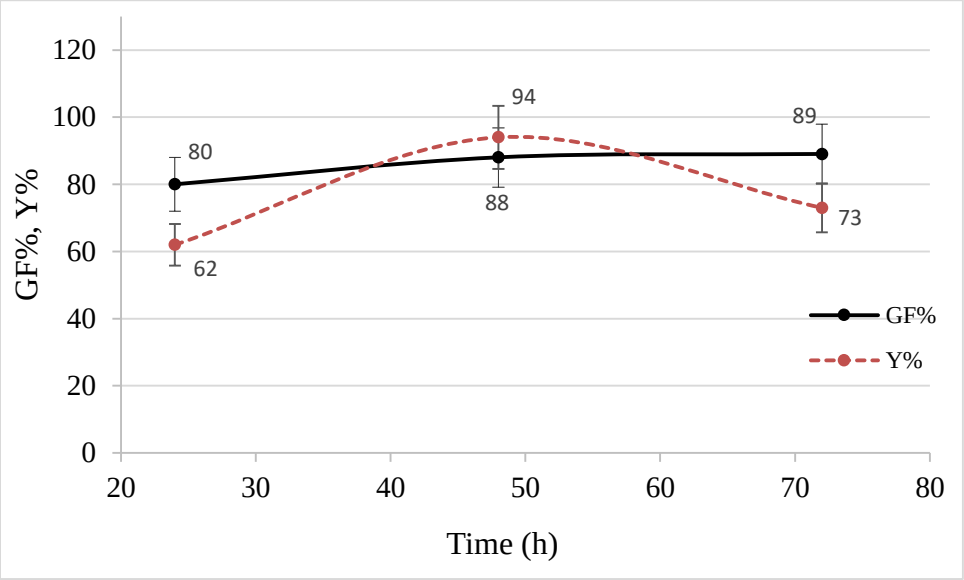

Figure 4.20. Effect of time on pullulan/PNVI cryogels gel fraction percentage (GF%) and yield percentage (Y%) using 6.62 M NVI, 0.76 M APS, 7.00 M ECH and 40 (g/L) pullulan under -18°C

4.3.2 Elemental Analysis

Elemental analysis of some available cryogel samples were carried out for finding H%, N% and C% values of the gels prepared under different conditions. Yield percentage of each sample is calculated using Equation (4.3). PNVI content is calculated by using N% reported by elemental analysis results using Equation (4.2).

PNVI% values agree with Y% obtained by gravimetric calculation. H%, N%, C% values, gel fraction percentage and PNVI content percentage of Sample 2, 20, 13, 25, 27 and 24 are reported in Table 4.2. N% values in the samples can be a proof of amine group presence in cryogels structure.

It can be followed from the elemental analysis reported in the table that Sample 2, crosslinked pullulan cryogel, has no nitrogen in its structure since there is no monomer used to prepare the pullulan gel. As it is showing in table, when monomer exist in the reaction medium, N% content, PNVI% content, yield percentage of the obtained gels

increase by increasing NVI under constant reaction condition, for instance Sample 25 (pullulan= 40 (g/L), NVI=0.44 M, N%=9.35, PNVI%=31.4, Y%=40) and Sample 27 (pullulan= 40 (g/L), NVI=6.62 M, N%=19.57, PNVI%=65.7, Y%=94). Sample 27 comparing to Sample 24, same amount of pullulan and NVI are used under same reaction condition but different ECH amount. Elemental analysis for Sample 27 (pullulan= 40 (g/L), NVI=6.62 M, ECH=7.00 M, N%=19.57, PNVI%=65.7, Y%=94) and Sample 24 (pullulan= 40 (g/L), ECH=0.70 M, NVI=6.62 M, N%=19.83, PNVI%=66.6, Y%=97) showing similar N% content and PNVI% content, since monomer amount used to prepare the gel was same for both samples.

Crosslinked PNVI cryogel, Sample 20 (NVI=3.31 M, ECH=7.00, N%=16.94, PNVI%=55.2, Y%=90) in compare to PNVI cryogel Sample 13 (NVI=3.31 M, N%=19.09, PNVI%=64.1, Y%=100), PNVI content is less. Higher PNVI% in PNVI cryogel, Sample 13, can explain good gel formation ability of NVI monomer under freeze thaw reaction without need of using any crosslinking agent. Good gel formation ability of NVI may explain hydrogen bonding formation through amine groups exist in imidazole ring.

Table 4.2. Elemental analysis results (N%, C%, H%), gel fraction percentage (GF%) and PNVI content percentage (PNVI%) of cryogels

No	[Pullulan] (g/L)	[NVI] M	[ECH] M	C%	H%	N%	Y%	PNVI%
2	40.0	-	7.00	39.32	6.59	-	32	-
20	-	3.31	7.00	33.60 28.24 37.09	6.25 5.44 6.14	16.44 16.15 16.94	90	55.2
13	-	3.31	-	38.08	5.84	19.09	100	64.1
25	40.0	0.44	7.00	19.69	5.16	9.35	40	31.4
27	40.0	6.62	7.00	44.78	6.48	19.57	94	65.7
24	40.0	6.62	0.70	42.15	6.00	19.83	97	66.6

4.3.3 FTIR-ATR Analysis

The FTIR-ATR spectrum of ECH, pullulan and pullulan/PNVI cryogel is given in Figure 4.21 (a), (b) and (c), respectively. In the FTIR-ATR spectrum of pullulan, at 3310 cm^{-1} the O-H stretching vibrations are observed. The C-H vibrations appear at 2930 cm^{-1} and the C-O stretching vibrations of the glycosidic and etheric bonds of the polymer are observed at 1148 cm-^{1}, 1078 cm^{-1}, 995 cm^{-1} and 929 cm^{-1}. The FTIR-ATR spectrum of PNVI homopolymer gives peaks at 3143 cm^{-1}, 2922 cm^{-1}, 2848 cm^{-1} are due to N-H stretching and C-H stretching vibrations respectively. The stretching vibration of C=N, C-N and C=C of the imidazole ring appear at 1641 cm^{-1} and 1556 cm^{-1}. The C-H vibrations are observed at 1490 cm^{-1} and 1300 cm^{-1} region [44]. In the spectrum of epichlorohydrin (ECH) the C-H vibrations appear between 2900 cm^{-1} to 3100 cm^{-1}. C-O stretching vibrations are observed at 1000 cm^{-1} to 1600 cm^{-1} and C-C stretching vibrations are observed at 700 cm^{-1} to 1000 cm^{-1}. Therefore, formation of pullulan/PNVI cryogel is confirmed by FTIR-ATR analysis. In the spectrum of pullulan/PNVI cryogel characteristics of ECH, pullulan and PNVI can be observed, Figure 4.21.

In addition to C-H vibrations due to crosslinking are observed at 2848 cm^{-1} and 3110 cm^{-1}, glycosidic and etheric bonds of pullulan in the region 1250-900 cm^{-1}, characteristic C=N stretching bonds of PNVI can be observed at 1738-1658-1645-1542-1419 cm^{-1} region, C-N stretching bond of PNVI can be observed at 1258 cm^{-1} region in cryogel sample spectra.

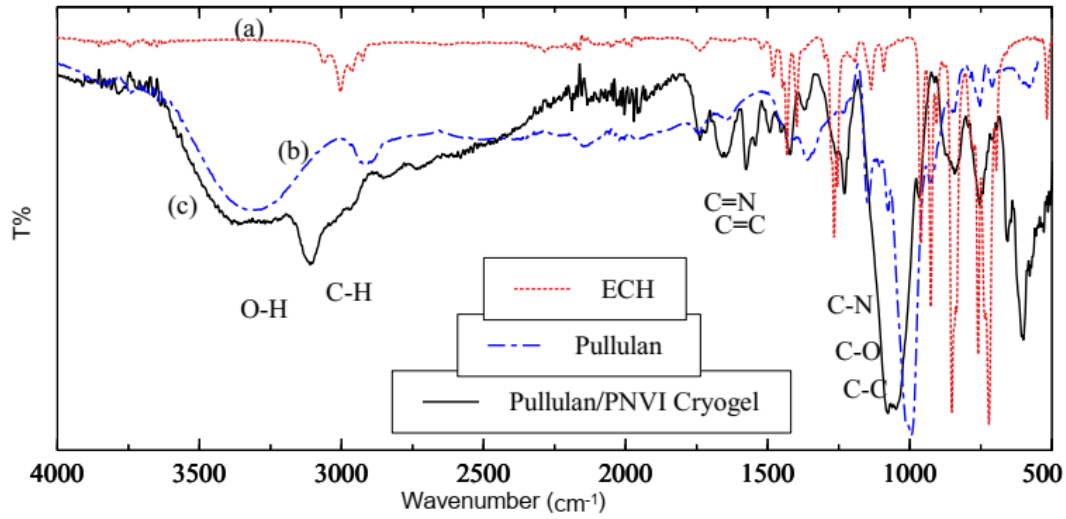

Figure 4.21. FTIR-ATR spectrum of (a) ECH, (b) pullulan and (c) pullulan/PNVI cryogel samples

4.3.4 Swelling Test

The prepared cryogels swelling property is tested at room temperature in aqueous medium. For swelling test 0.05 gram of each sample was weighed and swollen in distilled water. Weight of swollen samples were taken every 5 minutes (min) for 24 h. Swelling percentage of cryogels reported in Table 4.1 were calculated using Equation (3.1). Digital picture of the one pullulan cryogel and one pullulan/PNVI cryogel before and after water absorption (prepared by the thesis author in Eastern Mediterranean University/Physical Chemistry Laboratory) is shown in Figure 4.22.

Figure 4.22. (a) Freeze dried pullulan cryogel, (b) swollen pullulan cryogel, (c) freeze dried crosslinked PNVI cryogel, (d) swollen crosslinked PNVI cryogel, (e) freeze dried PNVI cryogel, (f) swollen PNVI cryogel, (g) freeze dried pullulan/PNVI cryogel, (h) swollen pullulan/PNVI cryogel

4.3.4.1 Effect of Preparation Conditions on the Swelling Capacity of Pullulan-ECH Cryogel

Swelling percentage equilibrium percentage of each crosslinked pullulan cryogel sample weas calculated using Equation (3.1). Effect of the amount of crosslinking agent on crosslinked pullulan cryogels is shown in Figure 4.23. Under constant reaction condition, ECH concentration is changed in Sample 1, 2 and 3 reported in Table 4.3. Increasing ECH concentration from 0.70 M to 7.00 M and to 14.0 M, Sample 1, 2 and 3 respectively, brings about decreasing swelling percentage

equilibrium as 425%, 210% and 150% respectively due to increasing crosslinking density with increasing ECH concentration, tighter network structure limits the elasticity of the hydrogel [26].

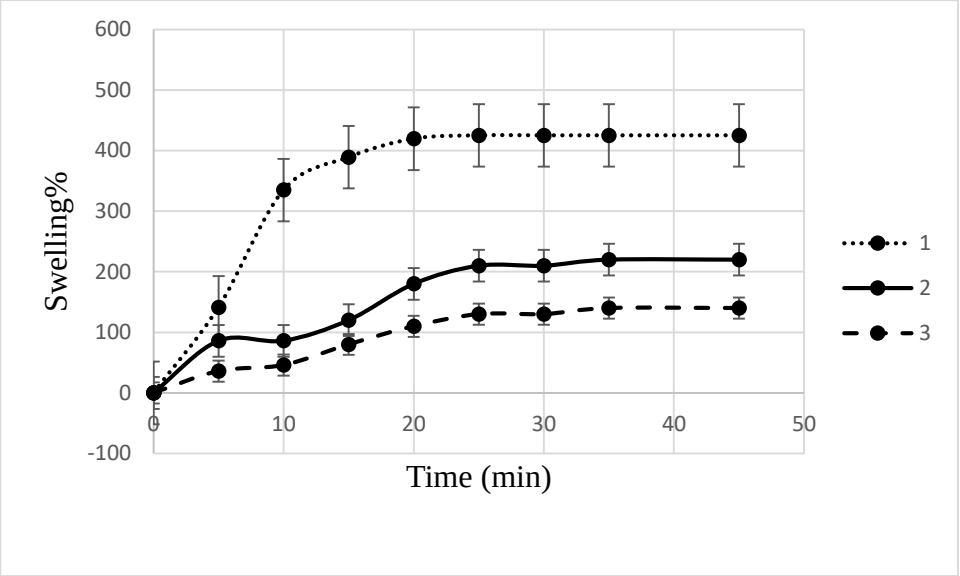

Figure 4.23. ECH concentration effect on crosslinked pullulan cryogel swelling percentage (S%)

Effect of reaction duration on the swelling behaviour of pullulan cryogels is shown in Figure 4.24. Gelation reaction duration is changed as 24 h, 48 h and 72 h for Sample 8, 2 and 9 respectively under constant reaction condition. Swelling percentages of Sample 8, 2 and 9, are obtained as 240%, 210% and 250%, respectively. It can be followed from Figure 4.24 that there is no significant change at equilibrium swelling percentage values by increasing reaction duration. This results indicates that reaction duration does not affect the crosslinking density of the pullulan cryogels formed.

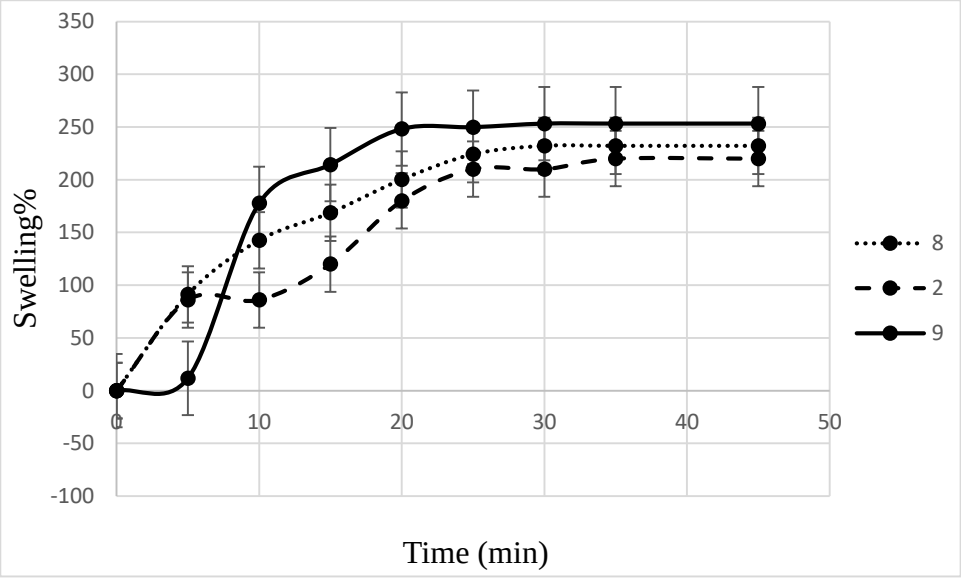

Figure 4.24. Time effect on crosslinked pullulan cryogel swelling percentage (S%)

Effect of reaction temperature on crosslinked pullulan cryogels is shown in Figure 4.25. Under constant reaction condition, reaction gelation temperature is changed as (-18°C, -22°C and -24°C) for Sample 2, 6 and 7, respectively. Swelling percentage of Sample 2, 6 and 7 are reported as 210%, 208% and 212%, respectively. It can be followed from figure that by decreasing reaction temperature, swelling percentage equilibrium of samples are not affected significantly.

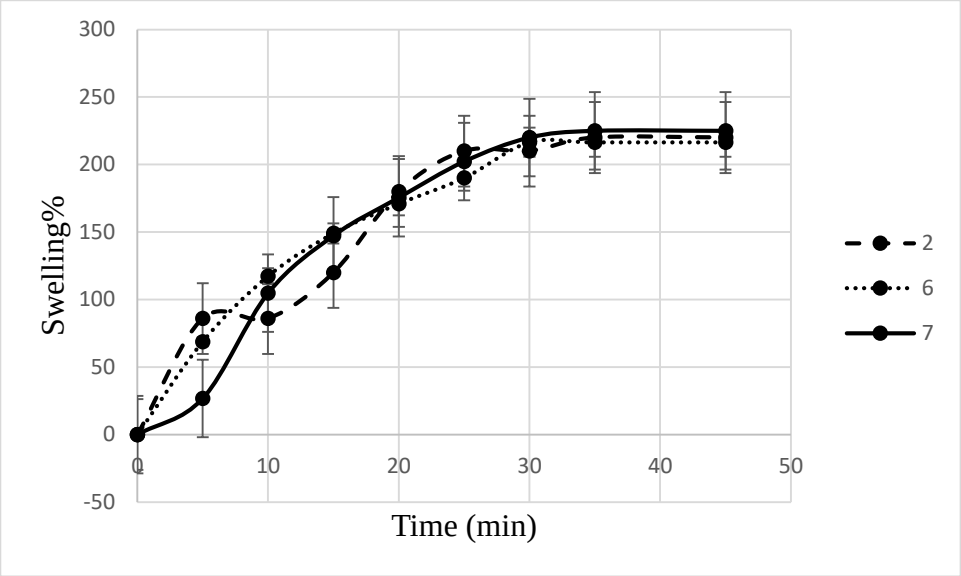

Figure 4.25. Temperature effect on crosslinked pullulan cryogel swelling percentage (S%)

Table 4.3. Effect of reaction conditions on equilibrium swelling capacity of pullulan cryogel

No	[Pullulan] (g/L)	[ECH] M	Temp	Time (h)	S%
1	40.0	0.70	-18	48	425
2	40.0	7.00	-18	48	210
3	40.0	14.0	-18	48	150
4	20.0	7.00	-18	48	180
5	10.0	7.00	-18	48	150
6	40.0	7.00	-22	48	208
7	40.0	7.00	-24	48	212
8	40.0	7.00	-18	24	240
9	40.0	7.00	-18	72	250

4.3.4.2 Effect of PNVI Cryogel Reaction Condition on Swelling Percentage

Effect of crosslinking agent on PNVI cryogels is shown in Figure 4.26. Under constant reaction condition, ECH concentration is changed in Sample 13, 19, 20 and 21. There is no crosslinking agent used to prepare cryogel Sample 13. ECH concentration used in Sample 19, 20 and 21 are 0.70 M, 7.00 M and 14.0 M respectively. As it can be followed from figure, there is no significant change in swelling percentage of Sample 19, 20 and 21 by increasing ECH concentration from 0.70 M to 14.0 M indicating that

NVI concentration is the limiting factor in the gel formation. There is not enough NVI in the medium to form crosslinking bond with excess ECH. However, there is an increase in swelling percentage equilibrium when crosslinking agent is used to prepare cryogel (Sample 19, 20 and 21, S%=270) in compare to Sample 13 (S%=240) that there is no crosslinking agent used in gelation reaction medium. By adding crosslinking bonds to PNVI structure, hydrophile (–OH) groups are adding to PNVI structure, this structure change in PNVI gel leads to higher water absorbability.

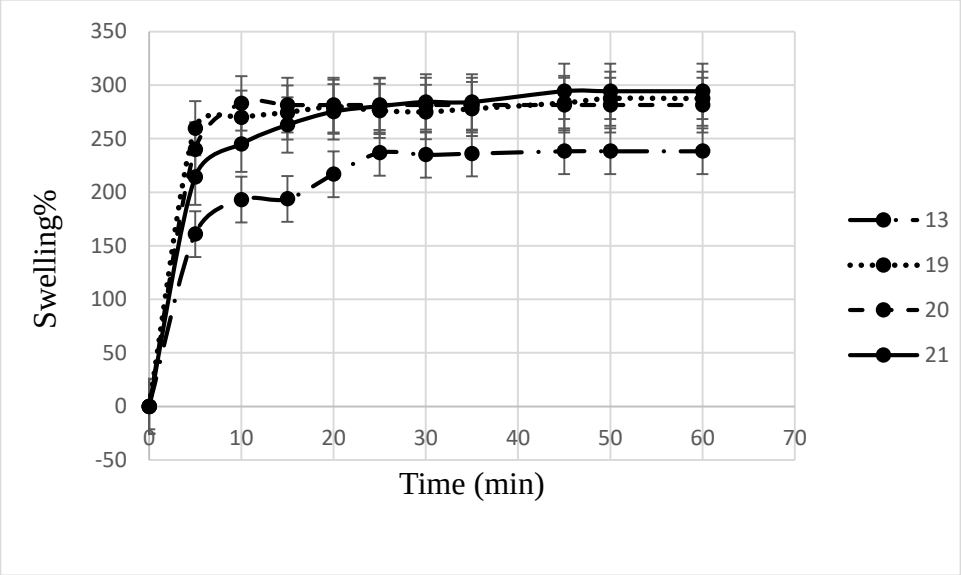

Figure 4.26. ECH concentration effect on PNVI cryogel swelling percentage (S%)

Effect of initiator on PNVI cryogels is shown in Figure 4.27. Under constant reaction condition, APS concentration is changed as 0.38 M, 0.76 M and 1.52 M for Sample 12, 13 and 14, respectively. It can be followed from the figure that by increasing APS amount from 0.38 M to 0.76 M there is an increase at S% from 130% to 240% and increase in yield percentage form 80% to 100%. Swelling percentage yield shown by Sample 13 is S%=240 when APS=0.76 M. Using APS concentration more than 0.76 M lead to less yield percentage (Y%=44) and lower swelling percentage (49%) (Sample 14). Higher APS concentration to a critical concentration causes increase in

polymer formation and increase in yield percentage, decrease in crosslinking probability and increase in swelling percentage.

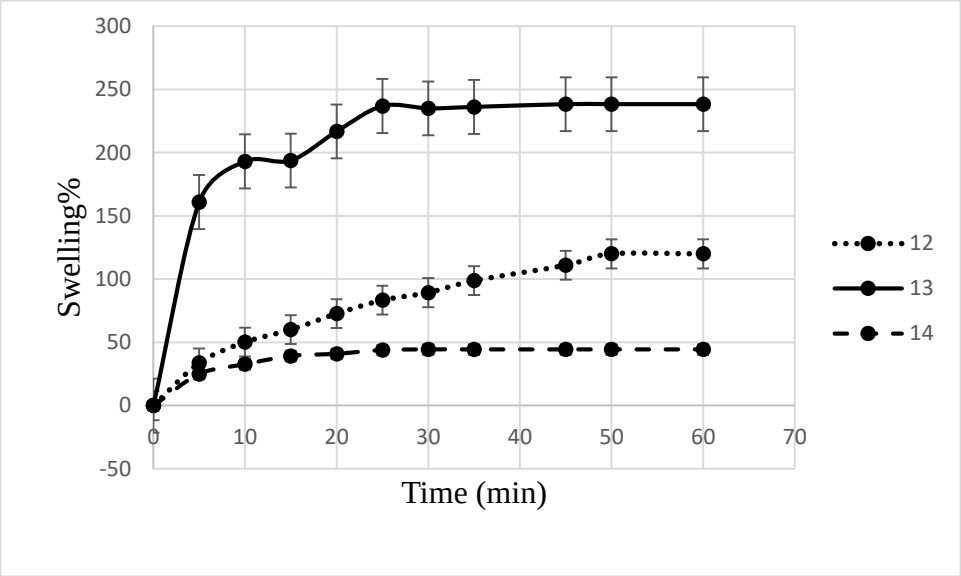

Figure 4.27. APS concentration effect on PNVI cryogel swelling percentage (S%)

Effect of reaction duration on PNVI cryogels is shown in Figure 4.28. Under constant reaction condition, reaction gelation duration is changed as 24 h, 48 h and 72 h for Sample 17, 13 and 18, respectively. Swelling percentage of Sample 17, 13 and 18 are reported as 270%, 240% and 55%, respectively. It can be followed from the figure that by increasing reaction duration swelling percentage equilibrium of samples decreases. Higher probability of dipole-dipole interaction by decreasing temperature can explain decline in swelling percentage.

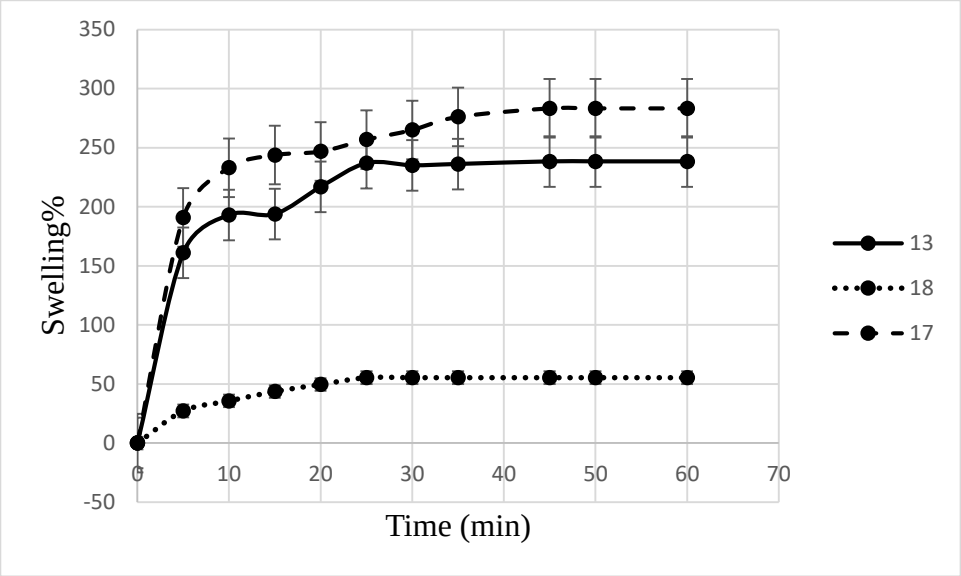

Figure 4.28. Time effect on PNVI cryogel swelling percentage (S%)

Effect of reaction temperature on PNVI cryogels is shown in Figure 4.29. Under constant reaction condition, reaction gelation temperature is changed as (-18°C, -22°C and -24°C) for Sample 13, 15 and 16, respectively. Swelling percentage value of Samples 13, 15 and 16 are reported as 240%, 42% and 45%, respectively. It can be followed from the figure that by decreasing reaction temperature, swelling percentage equilibrium of samples significantly decreases when the temperature decreases from -18°C to -22°C. Further decrease from -22°C to -24C does not have an effect on the swelling capacity.

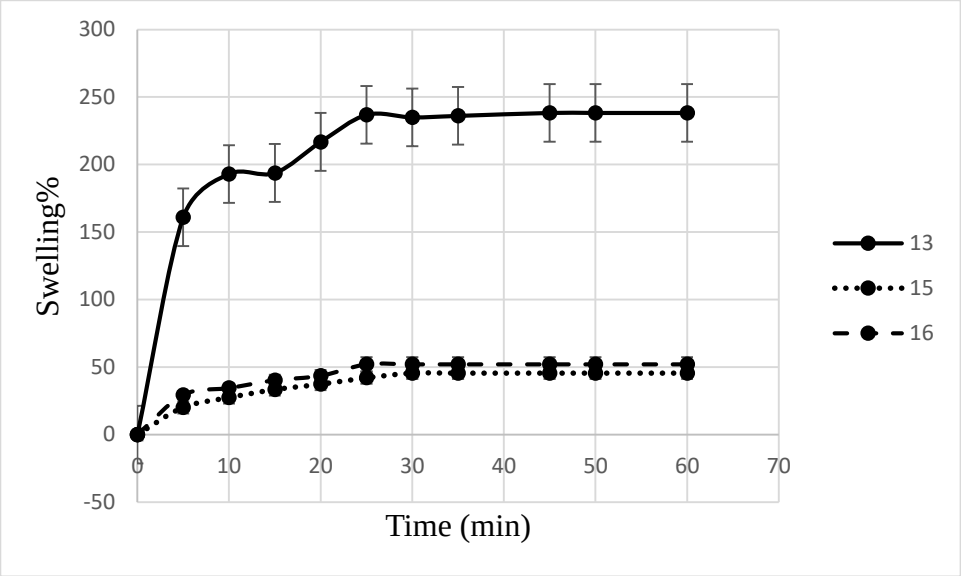

Figure 4.29. Temperature effect on PNVI cryogel swelling percentage (S%)

Table 4.4. Effect of reaction conditions on equilibrium swelling capacity of PNVI cryogel

No	[NVI] M	[APS] M	[ECH] M	Temp	Time (h)	S%
10	6.62	0.76	7.00	-18	48	270
12	3.31	0.38	-	-18	48	130
13	3.31	0.76	-	-18	48	240
14	3.31	1.52	-	-18	48	49.0
15	3.31	0.76	-	-22	48	42.0
16	3.31	0.76	-	-24	48	45.0
17	3.31	0.76	-	-18	24	270
18	3.31	0.76	-	-18	72	55.0
19	3.31	0.76	0.70	-18	48	270
20	3.31	0.76	7.00	-18	48	270
21	3.31	0.76	14.0	-18	48	270
37	0.44	0.76	7.00	-18	48	270

4.3.4.3 Effect of Pullulan/PNVI Cryogel Reaction Condition on Swelling Percentage

Effect of pullulan concentration on pullulan/PNVI cryogels is shown in Figure 4.30. Under constant reaction condition, pullulan concentration is increased as 40 (g/L), 100 (g/L) and 200 (g/L) in Sample 27, 28 and 29. By increasing pullulan concentration as 40 (g/L), 100 (g/L) and 200 (g/L) swelling percentage equilibrium decreases as 620%,

230% and 100%, respectively due to more probability of H-bonding in the reaction medium in between pullulan OH groups and NVI amine groups and formation of a tighter network.

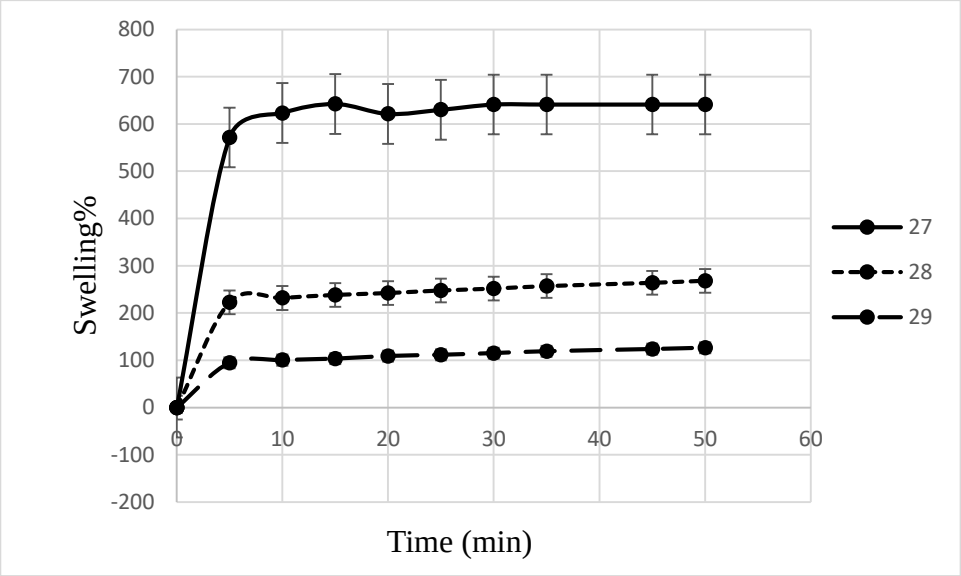

Figure 4.30. Pullulan effect on pullulan/PNVI cryogel swelling percentage (S%)

Effect of monomer concentration on pullulan/PNVI cryogels is shown in Figure 4.31. Under constant reaction condition, monomer concentration is increased as 0.44 M, 3.31 M and 6.62 M in Samples 25, 26 and 27. According to the figure, there is no significant change on swelling percentage of Sample 25, (S%=300) and Sample 26, (S%=290) under constant, pullulan 40 (g/L), APS 0.76 M and ECH 7.00 M amounts and -18°C reaction temperature for 48 h reaction duration. After increasing 3.31 M monomer concentration to 6.62 M, swelling percentage of cryogels increased to 620%. One reason for this behavior could be insertion of a greater fraction of –OH functionalities into the network structure due to the crosslinking reaction between NVI and ECH.

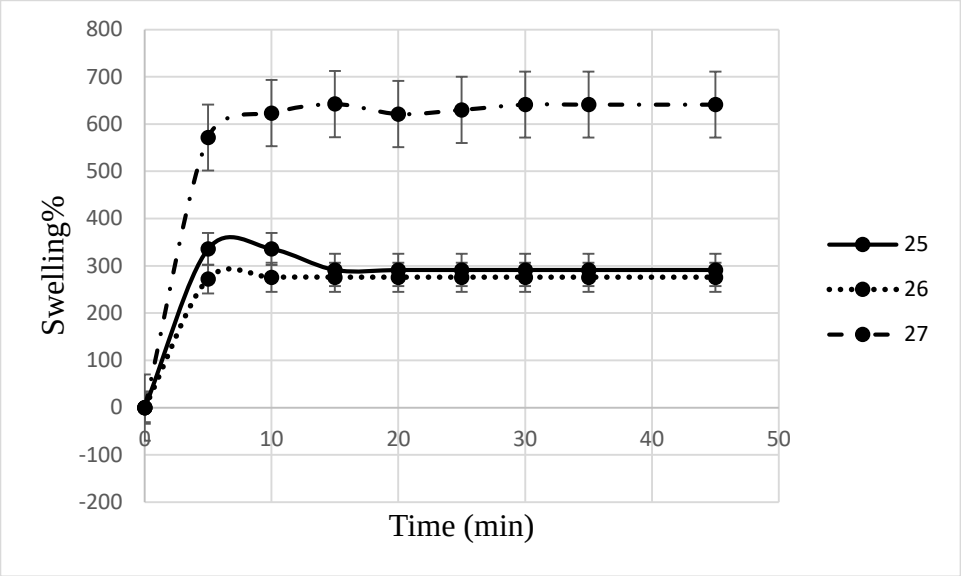

Figure 4.31. Monomer effect on pullulan/PNVI cryogel swelling percentage (S%)

Effect of initiator on pullulan/PNVI cryogels is shown in Figure 4.32. Under constant reaction condition, APS concentration is changed as 0.38 M, 0.76 M and 1.52 M for Sample 30, 27 and 31 respectively. It can be followed from the figure that swelling percentage equilibrium point for Sample 27 is S%=620 when 0.76 M APS concentration and. By increasing APS amount from 0.38 M to 0.76 M, there is an increase at S% from 295% to 620%. Higher APS concentration to a critical concentration causes increase in polymer formation and increase in yield percentage, decrease in crosslinking probability and increase in swelling percentage.

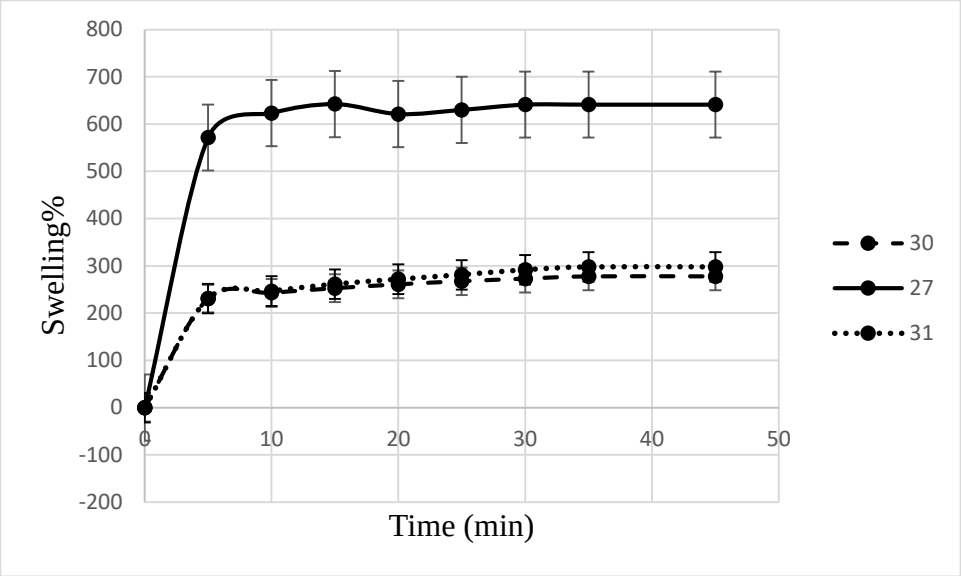

Figure 4.32. APS effect on pullulan/PNVI cryogel swelling percentage (S%)

Effect of crosslinking agent on Pullulan/PNVI cryogels is shown in Figure 4.33. Under constant reaction condition, ECH concentration is changed in Sample 24, 27 and 36 as 0.70 M, 7.00 M and 14.0 M respectively. As it can be followed from figure, there is an increase in swelling percentage of Sample 24, (ECH=0.70 M, S%=290) to Sample 27, (ECH=7.00 M, S%=620). Increases the amount of ECH more than 7.00 M there is no significant change in swelling percentage equilibrium, Sample 36 (ECH=14.0 M, S%=630). By adding crosslinking bonds to PNVI structure, hydrophile (–OH) groups are adding to PNVI structure, this structure change in PNVI gel leads to higher water absorbability. As long as crosslinking agent exist in the medium to form crosslinking bond, swelling percentage increases. When there is no more ECH in the medium but still active OH side of pullulan and amine groups of monomer, possibility of H-bonding increased causing decreased swelling percentage.

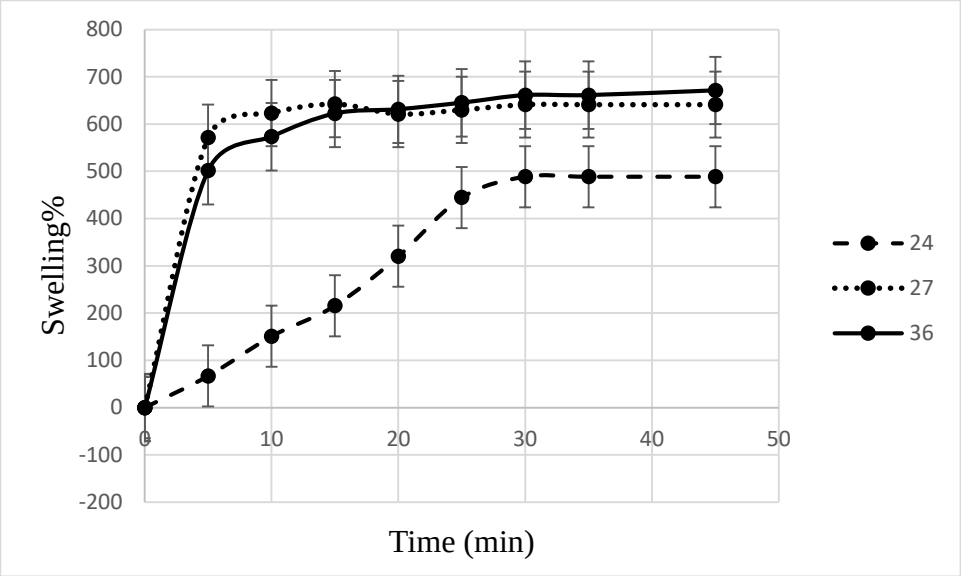

Figure 4.33. ECH effect on pullulan/PNVI cryogel swelling percentage (S%)

Effect of reaction duration on pullulan/PNVI cryogels is shown in Figure 4.34. Under constant reaction condition, reaction gelation duration is changed as 24 h, 48 h and 72 h for Sample 32, 27 and 33 respectively. Swelling percentage of Sample 32, 27 and 33 are reported as 290%, 620% and 300% respectively. It can follow from the figure that by increasing reaction duration from 24 h to 48 h swelling percentage equilibrium of samples increased from 290% to 620% respectively. After 48 h of gelation reaction duration swelling percentage decreased to 300%. Higher probability of H-bonding and dipole-dipole interaction formation by increasing time after 48 h can explain decline in swelling percentage of copolymer cryogel.

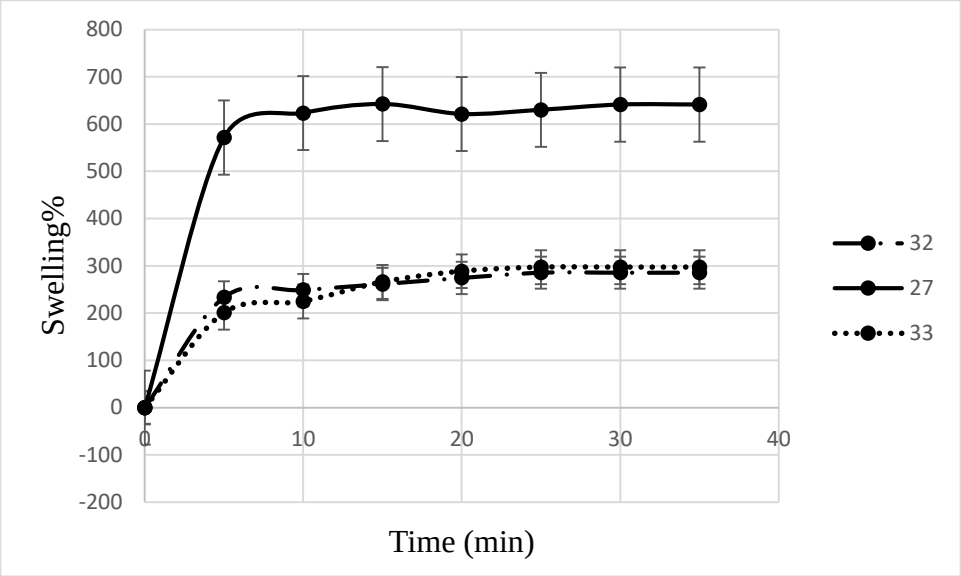

Figure 4.34. Time effect on pullulan/PNVI cryogel swelling percentage (S%)

Effect of reaction temperature on pullulan/PNVI cryogels is shown in Figure 4.35. Under constant reaction condition, reaction gelation temperature is changed as (-18°C, -22°C and -24°C) for Sample 27, 34 and 35 respectively. Swelling percentage of Sample 27, 34 and 35 are reported as 620%, 190% and 180% respectively. It can follow from the figure that by decreasing reaction temperature, swelling percentage equilibrium of samples decreased. Higher probability of H-bonding dipole-dipole interaction formation by decreasing temperature can explain decline in swelling percentage. Effect of reaction temperature on PNVI cryogels shows similar behaviour.

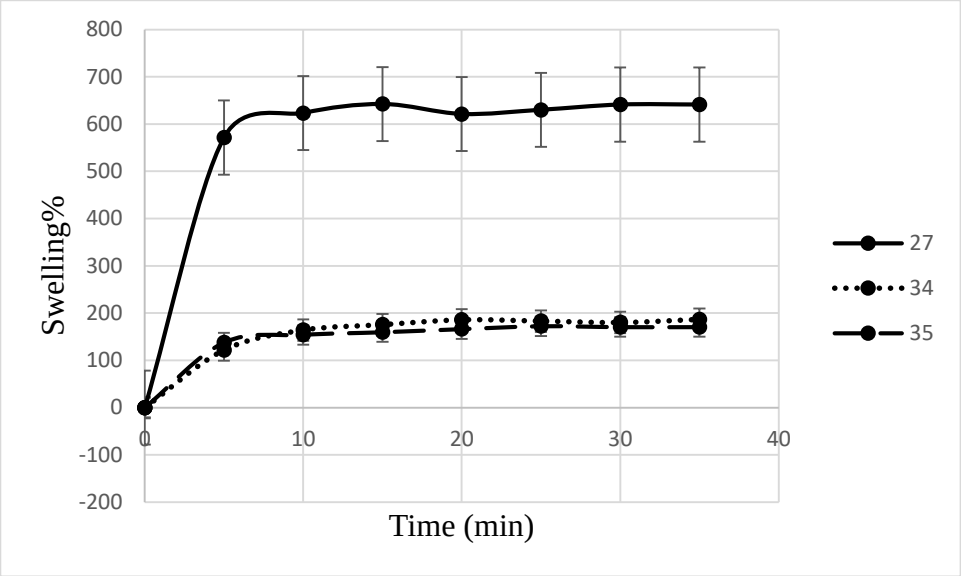

Figure 4.35. Temperature effect on pullulan/PNVI cryogel swelling percentage (S%)

Table 4.5. Effect of reaction conditions on equilibrium swelling capacity of PNVI cryogel

No	[Pullulan] (g/L)	[NVI] M	[APS] M	[ECH] M	Temp	Time (h)	Observation
25	40.0	0.44	0.76	7.00	-18	48	300
26	40.0	3.31	0.76	7.00	-18	48	290
27	40.0	6.62	0.76	7.00	-18	48	620
28	100	6.62	0.76	7.00	-18	48	230
29	200	6.62	0.76	7.00	-18	48	100
30	40.0	6.62	0.38	7.00	-18	48	295
31	40.0	6.62	1.52	7.00	-18	48	300
32	40.0	6.62	0.76	7.00	-18	24	290
33	40.0	6.62	0.76	7.00	-18	72	300
34	40.0	6.62	0.76	7.00	-22	48	190
35	40.0	6.62	0.76	7.00	-24	48	180
36	40.0	6.62	0.76	14.00	-18	48	630

4.3.5 TGA Analysis

Thermal behaviour of Samples 2, 13, 20, 24, 25 and 27 was investigated by thermal gravimetry analysis and reported in Table 4.1. TGA thermograms of the analysed samples shown in Figure 4.36. TGA data for Samples 2, 13, 20, 24, 25 and 27 are also shown in Table 4.3. T_1 is water loss temperature (°C), T_2 is decomposition temperature (°C), W_1 is weight of water in sample (%), W_2 is weight of sample lost at

decomposition temperature (%), W_3 remained weight of sample after decomposition is complete (%). Water loss of samples started at 15°C for T_1. At T_1 sample lose (W_1%) is due to water lost. Decomposition temperature of each sample is shown as T_2°C and W_2% is the weight loss of samples after reaching decomposition temperature. All samples are completely decomposed at around 650°C by remaining W_3% of their initial weight. According to data reported in Table 4.3, both crosslinked PNVI (Sample 20) and PNVI (Sample 13) cryogels lose water at 220°C to around 25% of their weight. Decomposition temperature for PNVI samples is at 325°C. After being completely decomposed at around 650°C, 32% weight of crosslinked PNVI (Sample 20) and 28% weight of PNVI (Sample 13) cryogels remain.

Sample 27 and 24 are prepared under the same reaction conditions and only ECH different. Pullulan/PNVI cryogel, ECH=7.00 M (Sample 27) in compare to pullulan/PNVI cryogel, ECH=0.70 M (Sample 24) shows higher thermal stability. Complete decomposition of Sample 27 takes longer than Sample 24. Decomposition temperature for Sample 27 is 350°C and for Sample 24 is 325°C due to more crosslinking bond in Sample 27 than Sample 24.

In compare to crosslinked PNVI cryogel that 20% of its initial weight is water loses at 220°C (Sample 20), 5% of initial weight of crosslinked pullulan is water loses at 100°C (Sample 2). Crosslinked PNVI decomposition temperature (325°C) is higher than that of the crosslinked pullulan decomposition temperature (300°C). Around 650°C by termination of decomposition, 32% of crosslinked PNVI weight and 25% of pullulan weight is remained in the medium. This shows crosslinked pullulan (Sample 2) thermal stability is less than crosslinked PNVI (Sample 20). Pullulan (Sample 2)

decomposition temperature is similar to pullulan/PNVI, NVI=0.44 M (Sample 25) at T_2=300°C.

Sample 27 and 25 are prepared under the same reaction conditions only monomer concentration was different. Pullulan/PNVI cryogel, NVI=6.62 M (Sample 27) in comparision to pullulan/PNVI cryogel, NVI=0.44 M (Sample 25) shows higher thermal stability. Decomposition temperature for Sample 27 is 350°C and for Sample 25 is 300°C due to more monomer concentration causes more crosslinking bond and H-bonding in Sample 27 structure. By increasing monomer concentration in the medium number of active amine groups increases and H-bonding and dipole-dipole interaction probability increases. As long as enough ECH is available in the medium, increase in monomer concentration lead to an increase in probability of crosslinking [60].

Table 4.6. Parameter evaluated from TGA analysis and cryogels reaction conditions for sample 2, 13, 20, 24 and 25. T_1 is onset decomposition temperature (°C), T_2 is decomposition temperature (°C), W_1 is weight of water in sample (%), W_2 is weight of sample loss at decomposition temperature (%) and W_3 remained weight of sample after complete decomposition (%)

No	[Pullulan] (g/L)	[NVI] M	[ECH] M	T_1 (°C)	W_1%	T_2 (°C)	W_2%	W_3%
2	40.0	-	7.00	100	5	300	43	25
13	-	3.31	-	220	25	325	45	28
20	-	3.31	7.00	220	25	325	45	32
24	40.0	6.62	0.70	220	20	325	45	29
25	40.0	0.44	7.00	100	3	300	50	15
27	40.0	6.62	7.00	220	20	350	45	26

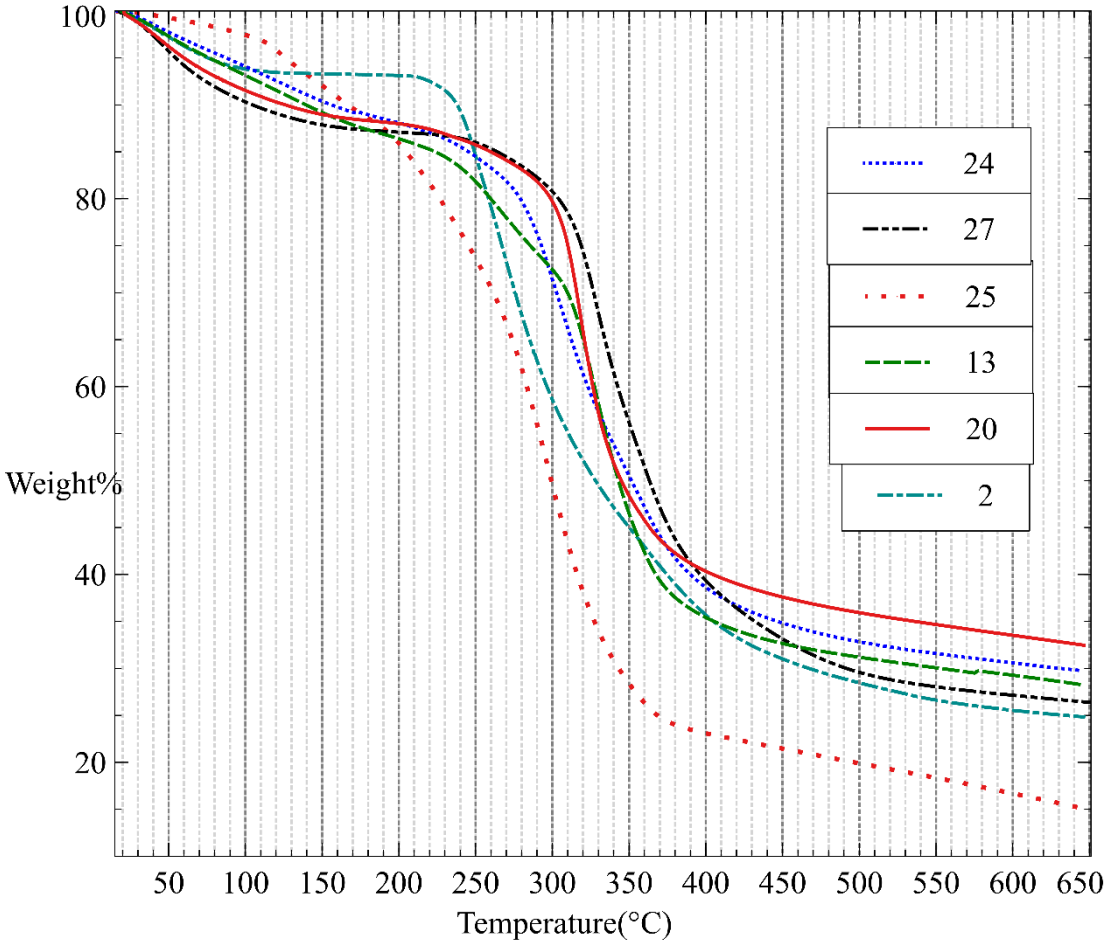

Figure 4.36. TGA curve of pullulan-ECH (ECH=7.00 M) (2), crosslinked NVI (ECH=7.00 M) (20), PNVI (13), pullulan/NVI cryogel (NVI=0.44 M and ECH=7.00 M) (25), pullulan/NVI cryogel (NVI=6.62 M and ECH=7.00 M) (27) and pullulan/NVI cryogel (NVI=6.62 M and ECH=0.70 M) (24)

4.3.6 X-Ray Diffraction

X-Ray diffraction patterns of Sample 24, 27, 2, 20, 13 and 25 are shown in Figure 4.37. A main diffraction peak appears at 2Θ=19.5° in the diffraction pattern of pullulan (Sample 2). The peak appears at 2Θ=22° in PNVI cryogel (Sample 13) and ECH crosslinked PNVI cryogel (Sample 20). Pullulan/PNVI cryogels, (Samples 24, 25 and 27) give a diffraction centered at 2Θ=23°. An analysis of crystallinity cannot be based on these findings as the cryogels may contain water trapped within the gel structure, which affect the crystallinity of the samples. An in depth analysis is needed in this regard [26,54,61,62].

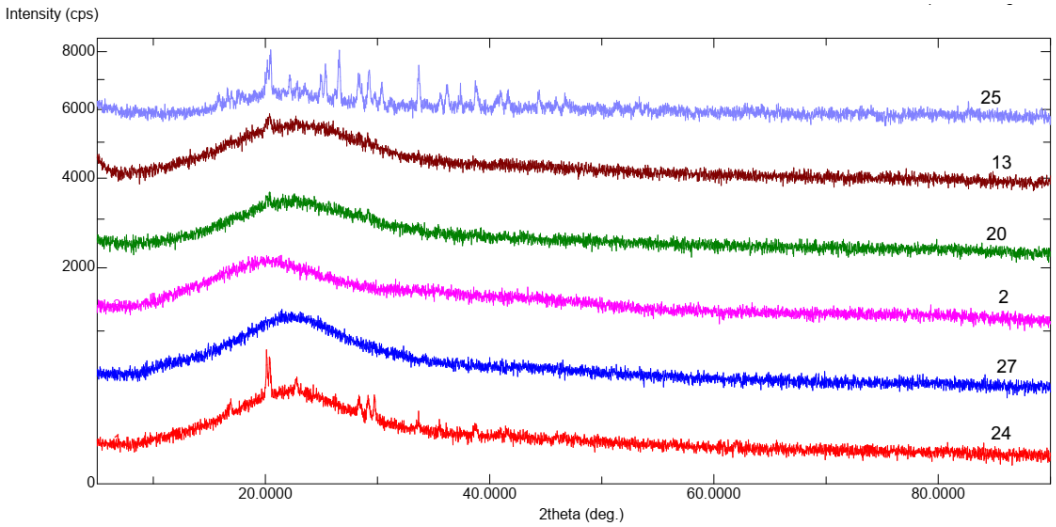

Figure 4.37. X-Ray diffraction of pullulan/PNVI cryogel NVI=6.62 M and ECH=0.70 M (24), pullulan/PNVI cryogel NVI=6.62 M and ECH=7.00 M (27), crosslinked pullulan cryogel (2), crosslinked PNVI cryogel (20), PNVI cryogel (13), pullulan/PNVI cryogel NVI=0.44 M and ECH=7.00 M (25)

4.3.7 SEM Analysis

Morphology of some cryogels, Sample (2, 20, 13, 25, 27 and 24) reported in Table 4.1, were examined by using scanning electron microscopy (SEM) analysis and are shown in Figure 4.38. Sample 2, is crosslinked pullulan cryogel, pullulan=40 (g/L) and ECH=7.00 M. Sample 20, is crosslinked PNVI cryogel, NVI=3.31 M, APS=0.76 M and ECH=7.00 M. Sample 13, is PNVI cryogel NVI=3.31 M, APS=0.76 M and no ECH is used in its gelation reaction. Sample 25, is pullulan/PNVI, pullulan=40 (g/L), NVI=0.44, ECH=7.00 M and APS=0.76. Sample 27, is pullulan/PNVI, pullulan=40 (g/L), NVI=6.62, ECH=7.00 M and APS=0.76. Sample 24, is pullulan/PNVI, pullulan=40 (g/L), NVI=6.62, ECH=0.70 M and APS=0.76. All samples were prepared under -18°C for 48 h of reaction duration.

Porous structure of all cryogels is visible in scanning electron microscopy images. According to SEM analysis, crosslinked PNVI cryogel (Sample 20, ECH=7.00,

S%=270) has less porosity than PNVI cryogel (Sample 2, ECH=0.00, S%=210) due to more crosslinking reaction in between amine groups on monomer structure and ECH.

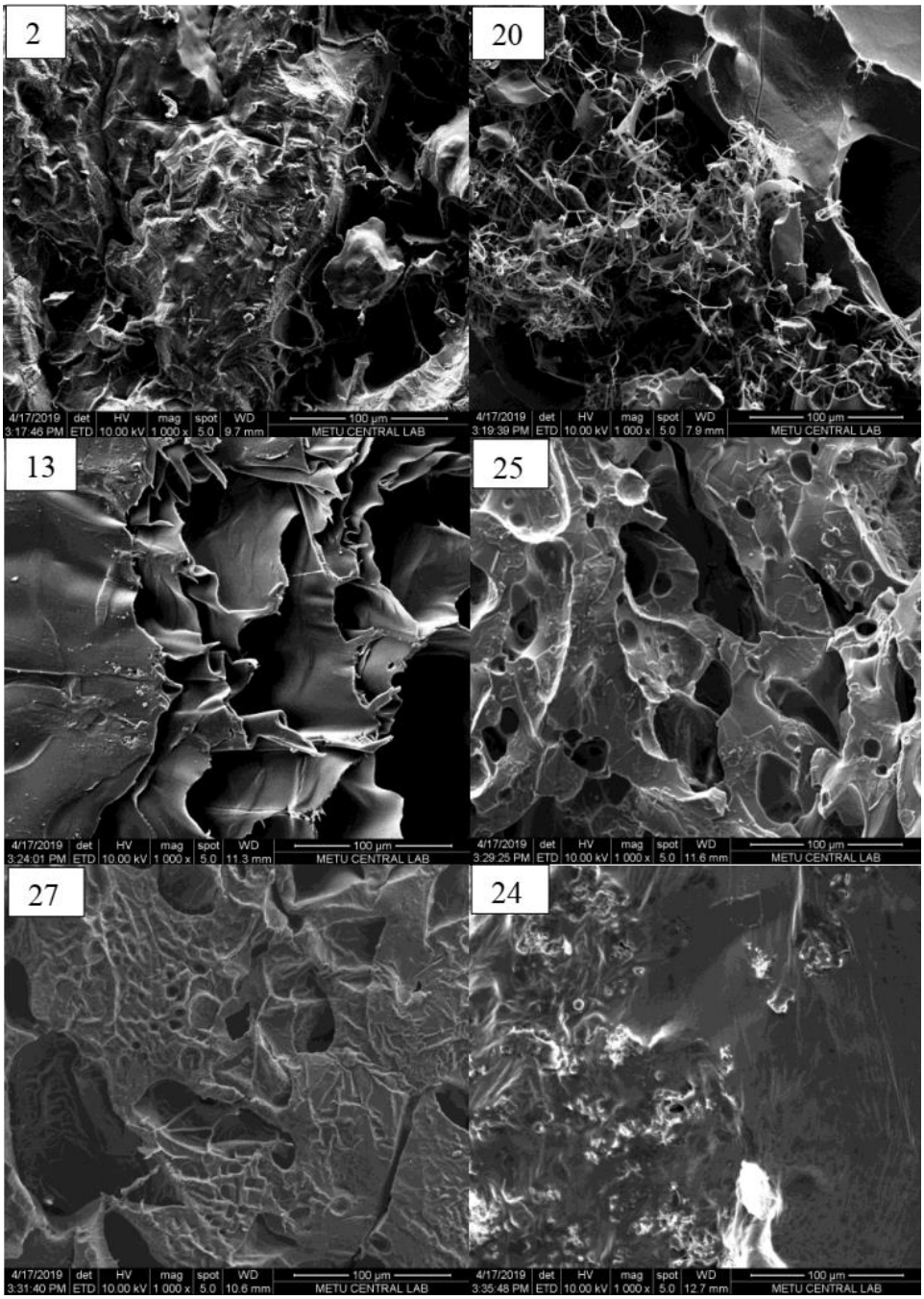

Figure 4.38. SEM analysis of pullulan (sample 2, pullulan=40 (g/L), ECH=7.00 M), crosslinked PNVI (Sample 20, NVI=3.31 M, ECH=7.00 M), PNVI (Sample 13, NVI=3.31 M, ECH=0.00 M), pullulan/PNVI cryogel (sample 25, pullulan=40 (g/L), NVI=0.44, ECH=7.00), pullulan/PNVI cryogel (sample 27, pullulan=40 (g/L), NVI=6.62, ECH=7.00), pullulan/PNVI cryogel (sample 24, pullulan=40 (g/L), NVI=6.62, ECH=0.70)

4.4 Conclusion

Cryogelation and cryopolymerization techniques have successfully been applied to prepare pullulan, PNVI and pullulan/PNVI cryogels. ECH has proven to be effective in crosslinking both pullulan and PNVI under cryogelation conditions. Furthermore, PNVI demonstrated cryogelation capability in the absence of any chemical crosslinker. NVI undergoing cryopolymerization initiated by APS, simultaneously forms polymer cryogel via dipole-dipole interaction and via H-bonding interaction between NVI functionalities and water molecules available in the medium. All cryogels formed exhibit a porous morphologies characteristic of cryogels. They are hydrogels having high swelling capacity in aqueous media.

Chapter 5

CONCLUSION AND FUTURE STUDIES

5.1 Conclusion

A neutral polysaccharide, pullulan, has been modified by grafting PNVI onto the polymer backbone in aqueous solution. The highest calculated grafting yield is G%=513. Pullulan-*graft*-PNVI copolymers obtained in this study are water soluble with potential biomedical applications such as acting as drug conjugate, gene carrier, or drug carrier. The graft copolymer synthesized gave complexes in aqueous acidic medium upon mixing with sodium tripolyphosphate and sodium citrate solutions. Hence, cationic nature has been imparted onto pullulan. The modified grafted pullulan showed antifungal and antibacterial activities due to presence of imidazole ring and amine group on its backbone. Having antifungal and antibacterial properties make the novel copolymer usable in medicine industry.

Epichlorohydrin crosslinked pullulan hydrogel proved to be suitable materials for PNVI grafting in aqueous acidic solution. The highest calculated grafting yield is 189%. The pullulan-ECH-*graft*-PNVI copolymers are superabsorbent hydrogels with equilibrium swelling capacities of 700% to 1300% in distilled water at room temperature and 6000% to 8500% in acidic solution in room temperature. Therefore they act as pH responsive hydrogels. Pullulan-ECH-*graft*-PNVI copolymers obtained in this study are cationic superabsorbent hydrogels.

Cryogelation and cryopolymerization techniques have successfully been applied to prepare pullulan, PNVI and pullulan/PNVI cryogels. ECH has proven to be effective in crosslinking both pullulan and PNVI under cryogelation conditions. Furthermore, PNVI demonstrated cryogelation capability in the absence of any chemical crosslinker. NVI undergoing cryopolymerization initiated by APS, simultaneously forms polymer cryogel via dipole-dipole interaction. All cryogels formed exhibit a porous morphologies characteristic of cryogels. They are hydrogels swelling in aqueous media.

5.2 Future Studies

1) Molecular weight and molecular weight distribution of soluble samples need further more detailed analysis.

2) Light scattering, radius of gyration analysis need to be done to find out their connections with grafting G%, molecular weight and its effect on antifungal and antibacterial activities.

3) Applications of hydrogels and cryogels should be studied. For example, these gels may act as metal or dye absorbents as well as drug release media.

4) Chemical structure of the PNVI cryogels should be studied in more detail to understand the participation of water molecules in the cryogel structure.

5) The relationship between pore size and pore morphology of the cryogels deserve further detailed investigation.

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