Pullulan/Poly(2-Hydroxyl Ethyl Methacrylate) Cryogels

Selma Ustürk

Submitted to the
Institute of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Chemistry

Eastern Mediterranean University
September 2019
Gazimağusa, North Cyprus

Approval of the Institute of Graduate Studies and Research

Prof. Dr. Ali Hakan Ulusoy
Acting Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. İzzet Sakallı
Chair, Department of Chemistry

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. Elvan Yılmaz
Supervisor

Examining Committee

1. Prof. Dr. Mustafa Gazi

2. Prof. Dr. Elvan Yılmaz

3. Assoc. Prof. Dr. Terin Adalı

ABSTRACT

Pullulan – polyHEMA cryogel systems were synthesized by using radical chain polymerisation with the aid of chemical crosslinkers. Pullulan- ECH-*graft*-polyHEMA cryogels and pullulan-ECH-*graft*-polyHEMA-EGDMA cryogels were prepared from various different concentrations of starting monomer; 2-hydroxy ethyl methacrylate (HEMA) and natural polymer; pullulan. For all the cryogel systems epichlorohydrin (EPC) was used as crosslinker and for some of them in addition to EPC , ethylene glycol dimethacrylate was used. For the initiation of polymerization, the redox couples of ammonium persulfate (APS) and N,N,N',N;-tetramethelen diamine (TEMED) were used. Swelling characteristics of the cryogel systems were analysed in three different conditions; acidic, neutral and basic conditions at 25 ℃. The concentrations of monomers and crosslinkers were altered to synthesize different cryogels with different swelling characteristic features. In addition, scanning electron microscopy (SEM) and FT-IR spectroscopy analyses were used for structural and morphological characteristic features determination. Based on dynamic swelling tests, it was found that pullulan-ECH-*graft*-polyHEMA-EGDMA cryogels bears the highest equilibrium swelling capacity characteristics with 97 %. These cryogels has got the highest degree of porosity among all the other types of cryogels.

Keywords: Pullulan- ECH-*graft*-polyHEMA cryogels, epichlorohydrin (EPC), ammonium persulfate (APS) and N,N,N',N;-tetramethelen diamine (TEMED).

ÖZ

Pululan – polyHEMA kriyojel sistemleri serbest radikal zincir polimerizasyonu ve çapraz bağlayıcı varlığında sentezlenmiştir. Monomer olarak 2-hidroxy etil metakrilat (HEMA) ve doğal bir polimer olan pululan kullanılarak değişik konsantrasyonlarda pullulan-ECH-*graft*-polyHEMA kriyojeller ve pululan-ECH-*graft*-polyHEMA-EGDMA kriyojeller hazırlanmıştır. Tüm kriyojel sistemleri için çapraz bağlayıcı olarak epiklorohidrin ve epiklorohidrinin yanında etilen glikol dimetakrilat da (EGDMA) kullanılmıştır. Polymerizasyon başlatıcı çifti olarak amonyum persülfat (APS) ve N,N,N',N;-tetramethilendiamin (TEMED) kullanılmıştır. Kriyojellerin karakterizasyonu için 25 ℃ de saf su içinde , asitli ve bazik sulu ortamda yürütülen dinamik şişme çalışmaları gerçekleştirilmiştir. Deneylerde monomer ve çapraz bağlayıcı miktarı değiştirilerek farklı şişme özelliği gösteren kriyojeller sentezlenmiştir. Bunun yanı sıra, yapısal ve morfolojik karakterizasyon için taramalı elektron mikroskobu (SEM) görüntüleri alınmış ve FT-IR analizleri yapılmıştır. Dinamik şişme çalışmaları sonucunda, en uygun şişme profili gösteren kriyojel sisteminin %97 denge su içeriği ile pullulan-ECH-*graf*t-polyHEMA-EGDMA kryojelleri olduğu görülmüştür. SEM görüntülerinde ise en yüksek miktarda por sayısı yine aynı tür kriyojel sistemlerinde gözlemlenmiştir.

Anahtar kelimler: pullulan-ECH-*graft*-polyHEMA kriyojeller, epiklorohidrin (EPC), amonyum persülfat (APS) ve N,N,N',N;-tetramethilendiamin (TEMED).

DEDICATION

I dedicate this thesis to my family

ACKNOWLEDGMENT

I would like to thank to my supervisor Prof. Dr. Elvan Yılmaz for her continuous support and great guidance.

I would also like to record my feelings to my close friends; Cahit Özbilenler, Arwa Abou Rajab, and Faisal Mustafa who motivate and support me during the progression of this thesis.

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LIST OF ABBREVATIONS

AIBN	Azobisisobutyronitrile
ATR	Attenuated Total Reflection
APS	Ammonium Per Sulphate
-co -	Copolymer
CTS	Chitosan
DMF	Dimethylformamide
ECH	Epichlorohydrin
EGDMA	Ethylene Glycol Dimethacrylate
FTIR	Fourier-Transform Infrared Spectroscopy
-g-	Graft Copolymer
GF	Gel Fraction
GMA	Glycidyl Methacrylate
HEMA	2-Hydroxyethyl Methacrylate
HLC	Human-Like Collagen
IUPAC	International Union of Pure and Applied Chemistry
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide
NMP	Nitroxide-Mediated-Polymerization
SEM	Scanning Electron Microscope
TEMED	*N*,*N*,*N*′,*N*′-Tetramethylethylenediamine
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy
UV	Ultraviolet
VIM	N-vinyl imidazole

Chapter 1

INTRODUCTION

Polymeric cryogels are gel systems produced in moderately frozen solutions or colloidal dispersions. Even though cryogels of polysaccharides such as chitosan, sodium alginate and their derivatives have been synthesized and characterized, pullulan is a relatively untouched polymer of natural origin in this regard. Poly(2-hydroxy ethyl methacrylate) proved to take part in cryogel formation in the presence of chitosan and glycidyl methacrylate and/or ethylene glycol dimethacrylate. Very recent investigations carried out in our laboratory proved formation of pullulan cryogels crosslinked by epichlorohydrin. Pullulan/poly(2-hydroxy ethyl methacrylate) cryogelation/cryografting system has not been studied before to the best of our knowledge. Hence, in this thesis pullulan/poly(2-hydroxy ethyl methacrylate) cryogel synthesis via free radical grafting of poly(2-hydroxy ethyl methacrylate) onto pullulan by ammonium persulphate initiation in the presence of crosslinkers epichlorohydrin and ethylene glycol dimethacrylate has been undertaken. The aim of the thesis is to optimize the cryogel formation conditions.. To fulfill these aims the effect of the concentration of pullulan, 2-hydroxy ethyl methacrylate and epichlorohydrin concentrations on the product yield and gel fraction was investigated. The effect of reaction time and temperature was studied as well.

The cryogels obtained were characterized by FTIR-ATR and SEM analyses. The swelling behaviour of the gels in aqueous solution was followed.

1.1 Cryogels

The term cryogel is derived from the Greek word “kryos”, which means ice or freezing. Cryogels are prepared either by using partially frozen monomeric solutions or polymeric solutions to obtain 3D matrices. Cryogels can be prepared from many polymers with a various source of origin. They can be prepared either from natural polymers or from synthetic polymers. In addition, chemically modified natural polymers can also be used to form cryogels. Polymer chains can be linked either with chemical crosslinkers or ionic crosslinkers to form a network like cryogels. Hydrophilic cryogels have macroporous structures and they can be prepared at very low temperature below 0°C. Cryogelation is based on the crystallization of a solvent in the gelation medium. This makes the main difference between the standard gel formation and cryogel formation. In standard gel formation, there is no phase transition for solvent. However, in cryogel formation since the solvent should be crystallized, there is phase transition (Bencherif et al 2013; Okay 2014).

Usually the polymers used in the cryogelation have high blood compatability, high water content , non toxicity and reusability. These features make them suitable for biomedical applications. They have broad applications compared to other types of gels. Cryogels can be formed easily compared to other types of gels. Transportation of chemicals in cryogels is easier due to macroporous structure of cryogels (Bakhshpour 2019).

1.2 Formation of Cryogels

Initially all the monomers or polymers should be dissolved in an appropriate solvent to obtain a polymeric solution. After the preparation of polymeric solutions, they should be placed into medium at a very low temperature (below the freezing point of the solvent). This enables the solvent to crystallize and the polymeric materials stay in a solution form and they form unfrozen liquid microphases. Following the completion of polymerization, the frozen solution is thawed at room temperature, and crystalline, frozen solvent molecules defrost. Once they defrost, the cryogel with high degree of porosity is formed.

In cryogel formation, solvents should be frozen at temperatures lower than the temperature, where they form crystalline structures. Although the frozen solution seems like it is a homogeneous frozen block, it is in fact heterogeneous. Along with the frozen crystalline solvent structures, they also contain unfrozen microphases, which are the polymeric structures. Agents required for the cryogelations are collected together in these unfrozen microphases and they will be concentrated to form cryoconcentration. Unfozen microphases take place only for very small volume of an entire cryogelation medium. Since the gel initiator agents are concentrated at the microphases, they initiate cryogelation in unfrozen microphases. Frozen crystalline solvent structures act as pore making agent in the cryogelation medium. Once the frozen solvent molecules defrost, they will form spaces in the cryogel. This will generate macropores in the structure of cryogels. The surface tension between the unfrozen microphases and frozen solvent enable smooth and circular pore formation in the structure of cryogels. During the freezing process, frozen solvent molecules will expand until they reach to the other solvent molecules. Once they are

placed at room temperature, solvents will melt and since there is surface tension around the microphases, these will hold the porous structure in place in a regular form (Figure 1) (Andaç & Denizli 2014).

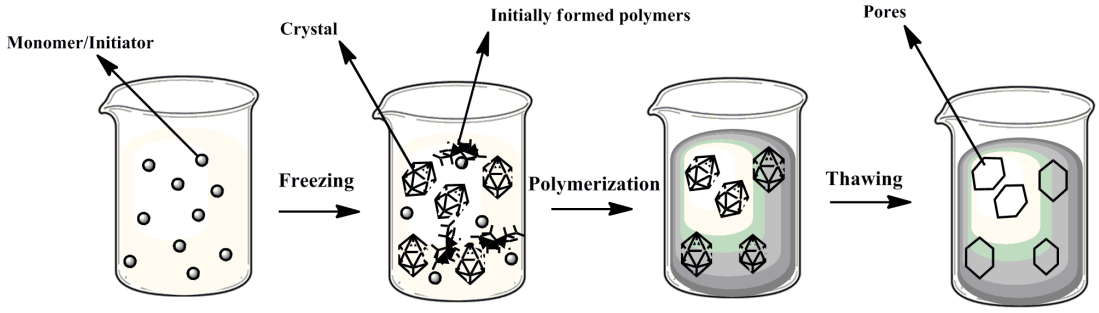

Figure 1:Shematic representation of cryogel formation.

1.2.1 Polymeric Cryogels Formed by Freeze Thawing of Colloid Solutions

Freeze-Thawing method for cryogelation was firstly used in food manufacturing and it was known as “kori-tofu” to produce tofu in Japan. To form heterogeneous mixtures colloidal solutions are mixed with chemical crosslinkers. For necessary period of time, cryogelation medium should be frozen and then it is defrosted to form pores. In order to change the size of the pores these freeze-thawing cycles can be increased. Generally, cryogels are prepared by using chemical crosslinkers, however, not all of them uses chemical crosslinkers. They can be prepared by using ionic crosslinkers, specifically when there are polymeric structures with charged groups or polar groups (Lozinsky 2018).

1.2.2 Cryogels Formed From Monomeric Solutions

There are three factors that must be considered when preparing cryogels from monomeric solutions. The first factor is to maintain conditions so that the solvent is crystallized. The second factor is to have a monomer with good solubility at low temperatures. If it has poor solubility, the monomer will precipitate out of the

polymeric solution. This will cause that in the gelation medium there will be very low amount of monomers available for cryogelation. The last factor is that the initiator system should be effective at low temperatures. Activation of initiator system is required for polymerization. Selection of initiator system plays a crucial role in cryogelation. Some initiator systems such as hydrogen peroxide and 2,2-azoisobutyronitrile require high temperatures for thermal decomposition to initiate polymerization. Such kind of initiator systems is not suitable for cryogelation. Redox initiation systems such as ammonium persulphate (APS) can be used to generate radicals at low temperatures (Nayak & Das 2018).

1.2.3 Cryogels Formation via Covalent Crosslinking Agent with High Molecular Weight Polymeric Solutions

Cryogel formation with high molecular weight polymeric solutions is dependent on two main factors. The first one is that the solubility of high molecular weight polymers in liquid microphases should be good enough for cryogelation. Otherwise, polymers may coagulate during cryogelation and it will be impossible to form cryogels. The second factor is that the polymer should have an optimum viscosity in solution. Due to high viscosity of high molecular weight polymers, they usually have low gelation time. If the polymer is so viscous, this means that mobility of the polymer chains is limited , therefore, it is less likely to interact with the initiator agents or gel forming agent. Eventually, this will not lead to the formation of cryogel. Even though higher molecular weight polymers usually have shorter gelation time, molecular weight of the polymer should be optimal to enable the segmental movements of polymer chains for the cryogel formation (Lozinsky 2018).

1.2.4 Cryogel Formation via Physical (Non-Covalent) Agent with Polymeric Solutions

Cryogels can be formed without requirement for chemical crosslinkers. PVA and self gel forming polymers are commonly used for non-covalent cryogel formation. 17% poly(acrylonitrile) solution in DMF/water mixture can be given as example for self-gelling polymers. This solution can form physical gel at -78°C and this physical gel is stable at room temperature. Therefore, poly(acrylonitrile) solution is suitable for cryogelation (Okay 2014).

1.3 Factors Affecting Cryogelation and Gel Fraction

Molecular weight of polymers, tacticity, type of functional groups, type of polymer, type of monomer, temperature, type of initiator, time, type of solvent, type of crosslinker are factors that affect cryogel formation and gel fraction.

Optimal molecular weight of polymers should be selected. Otherwise, high molcecular weight polymers have high viscosity, which prevents the segmental movements of polymer chains. This limits the cryogelation since the reactions between the initiator or the gel forming agents and the polymers will be limited. Tacticity of polymer can affect the liquid microphases of unfrozen polymeric material. Type of functional group will determine the type of weak interactions between functional groups of polymers. Polymers with self gelling ability may not be requiring crosslinking agent. In addition to this, a polymer with good polar functional groups may not require crosslinking agents or low gelation time. Monomers with low tendency for homopolymerization should be selected, otherwise, final product will be cryogel composed of only homopolymer.

Furthermore, careful determination of optimal gelation temperature is crucial. Solvent should form crystalline structures at gelation temperatures, without going

through glass transition state. Otherwise, cryogel will not form. Redox initiators are preferred as they initiate polymerization effectively at low temperatures. Some polymers requires more time for polymerization, others do not. Each solvent has different crystallization temperature. Optimal temperature should be preferred. Not all cryogelation medium requires crosslinkers. It is dependent on the application that polymer is required to be used (Niknia.,& Kadkhodaee 2017; Okay 2014).

1.4 Cryogels Morphology Effecting Factors

There are five main factors, which affects morphology of cryogels; The cooling rate during the freezing, the freezing temperature itself, frozen storage duration, the rate of the frozen samples heating for their thawing and the number of freeze-thawing cycles (Saylan & Denizli., 2019).

1.5 Gels, Hydrogels and Cryogels

There are huge differences between cryogels and the normal gels in terms of morphological structures.. It is clear that any gel formation, which requires thermal initiation will not be formed by cryogelation.

Hydrogels are network of polymers crosslinked via specific crosslinkers to prevent dissolution in an aqueous solutions. Hydrogels usually have hydrophilic characteristic features, which enables them to hold huge amount of water in their structures. Hydrogels can be physically cross-linked and chemically cross-linked gels. Physically cross-linked hydrogels can be formed by hydrogen bonding, ionic interactions, hydrophobic interaction and protein assisted interactions. Chemically cross-linked hydrogels can be formed by functional groups of cross-linker, radiation, radical polymerization and enzyme assisted crosslinking (Akhtar 2016).

Gels that are formed by freeze-drying method may resemble the similar properties with cryogels. For instance, they can swell in an appropriate solvent without showing any dissolution. However, compared to cryogels, freeze dried gels have limited applications Freeze dried gels are more suitable for thin and small materials such as thin films and beads. However, cryogels can also be used for bigger materials such as large cylinderic structures, large blocks or disks.

Cryogels are formed from heterogeneous mixtures and they are highly macroporous structures, so that the chemicals or solvents can be easily transported in the structure of cryogels. On the other hand, transport of solvents in the ordinary gels prepared from homogeneous mixture is more difficult and complicated (Rodríguez-Dorado 2019).

1.6 Natural Polymers

Natural polymers exist in the structure of living organisms.Rubber is the most common natural polymer. The molecular formula of C_5H_8 represents isoprene (IUPAC name is known as 2-methyl-1,3-buadiene). When the isoprene monomers react together, they will form polyisoprene and this polyisoprene is known as natural rubber. Natural rubber is less flexible compare to the other types of rubbers and they are sticky and less likely to be used in the daily life. Natural rubbers are subjected to sulfur between 30% and 50% to form harder rubbers via vulcanization procedure. These hard form of rubber can be used in the industrial products, specifically in the manufacturing of the car tyres. Natural polymers include proteins, cellulose, chitin, chitosan alginic acid, pullulan, gelatin, polylactic acid to name the most common ones.

1.6.1 Pullulan

Aureobasidium pullulans is a type of yeast and it is the member of fungus family. Pullulan is usually extracted from *A.pullulans* and it is a water soluble polymer given Figure 2. (Singh et al 2008).

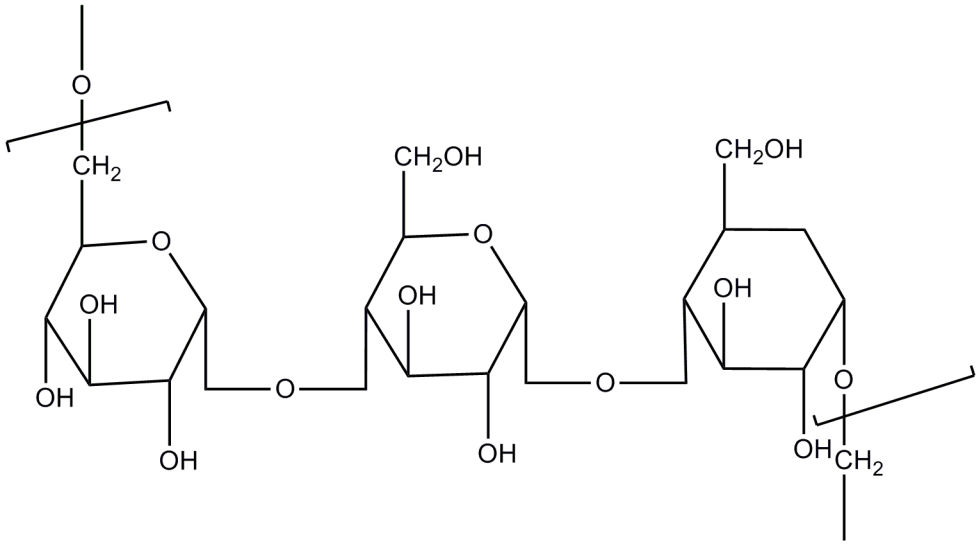

Figure 2: Chemical structure of pullulan with maltotriose as a repeating unit.

1.7 Synthetic Polymers

Synthetic polymers are synthesized in the laboratory by mimicking the natural polymers. Most of the synthetic polymers require hydrocarbon based sources in order to be synthesized and therefore petroleum is the rich source of origin for the synthetic types of polymers. First synthetic polymer is synthesized from phenol and formaldehyde to form bakalite. Polyethylene, polystyrene, polyethylene terapthalet, ethylene glycol, polyvinyl are the examples of synthetic polymers.

1.7.1 2-Hydroxyethyl Methacrylate (HEMA)

HEMA is a hydrophilic , synthetic polymer, which is frequently used as hydrogel.

There are commonly used two different single step synthesis pathway for HEMA. First one is production of HEMA from the reaction between methacrylic acid and

ethylene glycol, ethylene glycol dimethylacrylate was produced as side product. Second synthesis pathway involves reaction between methacrylic acid and ethylene oxide to form HEMA. Again EGDMA can be produce as side product (Montheard et al 1992). Chemical structure of HEMA contains nucleophilic hydroxyl group and electrophilic ester and alkene groups, which makes them suitable for radical polymerization (Figure 3)(Kemal et al 2011).

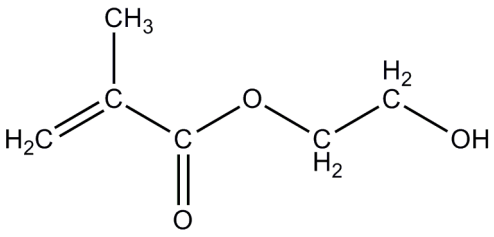

Figure 3: Chemical structure of HEMA.

Three are mainly three different ways to obtain polyHEMA from HEMA. The first one is bulk polymerization in the presence of crosslinker agent. Crosslinking of polymers in solution is a method to initiate polymerisation via the help of the crosslinkers at the same time as monomers polymerises.

The last method is the most frequently used to synthesis polyHEMA. This is beacause monomers are found in the polymerisation medium and this enable the polymerisation to be happen in a controllable manner.

PolyHEMA hydrogels are frequently used for contact lens production, implants, drug releasing systems and in many biomedical applications. polyHEMA hydrogels are preferential for these applications due to their biocompatibility, and hydrophilic, stable, soft, and permeable characteristics. In ordinary biological reactions they act as

inert material. They can resist against degradation in biological environments. Therefore, the body cannot absorb them. Due to their high stability they can be sterilized in autoclave. They can be shaped into various different shapes and forms. PolyHEMA gels can be synthesized in different ways to obtain gels with a different levels of porosity. Although polyHEMA gels have many desired properties, which makes them perfectly fit for many biological applications, they have limits in medical or biological applications due to their low mechanical strength and low biodegradability (Seidel & Malmonge2000).

1.8 Free Radical Polymerization

During the radical polymerization active centers of polymers have radical to further elongation of growing chain of polymers by reacting with more monomers. However, this require some kind of initiation mechanism to form radical on the monomers. These initiator mechanism can be chemical initiators such as ammonium persulfate , UV or thermal energy. Once the radical is formed on the monomers monomers are react with each other to form longer chain and each time one electron is transferred from the growing chain of polymer to the monomeric structure so that reactive center will be formed on the newly attached monomers for further polymerization (Ouellette & Rawn.,2015).

1.9 Initiator Systems

There are mainly 3 types of initiator systems; Thermal/UV initiation , redox initiation systems and initiation by radiation.

1.9.1 Thermal/UV initiation

Peroxides and organic azo compounds are easily form radicals in the presence of heat and they initiate polymerization (Figure 4).

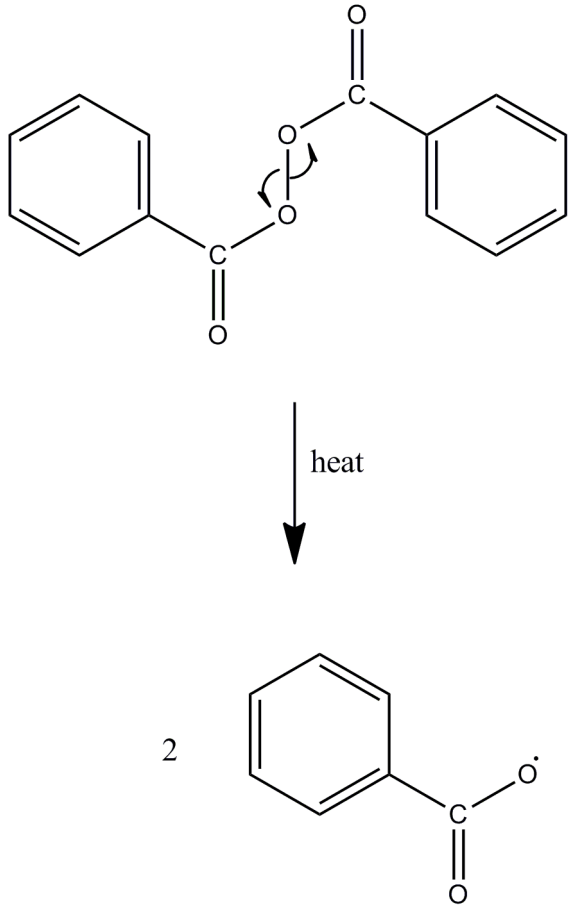

Figure 4: Thermal decomposition of benzoyl peroxide.

UV radiations can be used to accelerate the decomposition of the initiator system .

1.9.2 Redox Initiation Systems

Hydrogen peroxides and persulfates can easily form radicalic structure at room temperatures (Figure 4) (Kohut-Svelko 2009).

1.9.3 Initiation by Radiation

Using heat, light and highly energized radiations such as α,β,γ and x. Monomers such as styrene can be polymerize via the presence of light or heat. Therefore, it is a considerable issue that the monomers should be stored in the presence of inhibitor.

1.10 Graft Copolymerisation

Physical combination of two or more polymers are not always sufficient to synthesize novel gels or polymer networks with desired features. On the other hand, attachments of monomer units to the main polymer backbone to increase the desired features of the main backbone polymer or to introduce novel features to the polymer was possible by graft copolymerization. Presence of different polymer chains or monomer units on the main backbone polymer chains lead to the formation of copolymers.

CHAPTER 2

LITERATURE REVIEW

2.1 Pullulan Hydrogels

Sodium trimetaphosphate and epichlorohydrin are two mostly studied crosslinkers for pullulan. Pullulan undergoes crosslinking with sodium trimethaphosphate and epichlorohydrin to form hydrogels. Examples from the literature are summarized below. In one study, pullulan-sodium trimetaphospahte gels were investigated for cell proliferation. SEM images of these gels suggested that the surface of pullulan gels were smooth enough for seeding of smooth muscle cells onto them. Cell proliferation was monitored for about 1 week by using MTT assays. In vitro degradation was tested in pullulanase enzyme solution (Autissier et al 2006).

In another study, carboxymethyl pullulan was used to form microspheres by using sodium trimethaphosphate and epichlorohydrin as crosslinkers. Following the synthesis of microspheres, hydrophobic palmitoyl groups were attached onto pullulan gels to obtain anionic pullulan derivatives. Then, lysozymes were immobilized onto the synthesized pullulan derivatives and the effect of anionic hydrophobic groups was evaluated based on the enzyme adsorption and release studies (Mocanu et al 2004).

Pullulan can be used to prepare injectable biomaterials to repair tissue damages. For this purpose, pullulan and HLC (human-like collagen) with various different molecular weights were combined to form hydrogels. Pullulan was oxidized with sodium periodate. Aldehyde functional groups created on pullulan were reacted with amine groups of HCC and this caused the formation of hydrogels. This study proved that HLC/Pullulan hydrogels could be used as therapeutic agent to fill the soft tissues. Combination of HLC to pullulan hydrogels showed increased biocompatability, so that the gels will adhere to soft tissues without any inflammatory effects (Li et al., 2016).

Pullulan/poly(vinyl alcohol) hydrogels and cryogels were formed by initially oxidizing pullulan at carbo-6 position by using TEMPO, NaBr and sodium hypochlorite solution. The pH was adjusted to 10. Then, when the pH was maintained at 10, few drops of methanol was added to increase the acidity to pH 6.5. Oxidized pullulan was precipitated by ethanol. Following this step, oxidized pullulan was combined with poly(vinyl alcohol) to obtain hydrogels. Cryogel forms of these gels were prepared by freeze /thawing method. The significance of this hydrogels were found as when they were deformed they can reform their shapes and this indicates self-healing ability (Bercea et al 2018).

To obtain injectable and biodegradable pullulan gel, pullulan was functionalized with carboxymethyl group. Carboxymethyl pullulan was combined with tyramine and they were conjugated with chondroitin sulfate –tyramine in the presence of horseradish peroxidase for the generation of biomaterials such as cartilages. Successful cartilage designs were obtained from the mice experiments and

pullulan/chondroitin sulfate hydrogels showed sufficient cartilage regeneration (Chen et al 2016).

2.2 Graft Copolymers of Pullulan

Under nitrogen gas, methyl acrylate monomers were grafted onto pullulan to form water absorption resins. Although the percent grafting was affected due to high concentration of initiator and monomer, it was increased when the reaction time for grafting was increased. It was found that as the percent grafting increased, water absorbtion capacity was decreased.(We et al.2019).

Trimethyl silyl group was used as protection agent for pullulan to synthesize biodegradable graft polymer of poly(lactic acid) on to pululan. Compared to pullulan or poly(lactic acid) alone, the graft copolymer showed great hydrolytic biodegradability. In addition, the sugar components can be varied by changing either the amount of poly(lactic acid) or the L-lactide in the structure of poly(lactic acid) (ohya et al. 1998).

2.3 Poly-2-hydroxyethyl methacrylate (PolyHEMA) Cryogels

Poly(HEMA/GMA) membrane gels were synthesized by using uv irradiation to initiate photopolymerization. Chitosan was grafted on these polymers and for some of them Fe(III) ions were chelated. Chelation of Fe(III) ions on graft copolymer cable the immobilization of glucose oxidase enzymes. Following to enzyme immobilization the enzyme immobilization capacity of the graft copolymer was tested, optimal pH and temperature for immobilized enzymes were determined. (Yakup Arica et al 2006)

Poly(2-hydroxyethyl methacrylate-glycidyl methacrylate) cryogels were synthesized by using free radical polymerization following to the formation of cryogels. Peroxidase enzyme was immobilized onto the cryogels. These gels were used as absorbent for phenolic compounds and dyes and they were efficient tools for waste water treatment (Uygun et al 2018).

To lower the level of cholestrol, it was aimed to remove cholesterol molecules from hypercholesterolemic human plasma via the help of poly (HEMA-EGDMA). Anti-LDL molecules were attached to poly (HEMA-EGDMA) beads through protein. Beads were generated via suspension polymerization. Beads were repeatedly tested for 6 times and it was found that was no significant loss in the binding capacity to the cholesterol molecules (Yavuz and Denizli 2005).

In plastic syringe polyHEMA cryogel was initially formed. Carbodiimide was used for the anchorage of gelation molecules onto polyHEMA cryogels. PolyHEMA cryogels were used to extract fibronectin from human blood plasma. Studies showed that the capacity of fibronectin adsorption with gelatin-immobilised polyHEMA was higher than the adsorption capacity of PHEMA cryogels. It was found that these cryogels can be used for several times without affecting their capacity of adsorption. Elution of the adsorbed fibronectin was possible via the treatment with 2 M urea with 1M NaCl (Perçin et el 2013).

PolyHEMA cryogels are not only useful for biomedical applications, they can also be used for metal removal. N-vinyl imidazole monomer was used to synthesize poly (HEMA-VIM) cryogels by Tescin at al. Then, to increase the surface area of the cryogels obtained, the gels were prepared second time via suspension polymerization

to obtain poly (HEMA-VIM)/poly(HEMA) cryogels. In these kind of gels poly (HEMA-VIM) polymer were embedded in poly(HEMA) polymers. The effect of pH, metal ion concentration and adsorption time were studied for both poly (HEMA-VIM)/poly (HEMA) cryogels and poly (HEMA-VIM) cryogels to obtain a cryogels with optimum adsorption conditions. Pb^{+2} , Cd^{+2} ,Zn^{+2} and Cu^{+2} metal cations were tested and among all these four metal cations, it was found cryogels showed the highest binding capacity and affinity for Pb^{+2} ions. In addition, adsorption, desorption processes showed that gels can be used repeatedly without significant lost in adsorption capacity (Tekin et al 2011)

Chapter 3

EXPERIMENTAL

3.1 Materials

Pullulan (J&K, China), 2-hydroxy ethyl methacrylate (HEMA) (Sigma-Aldrich, Germany), N,N,N',N'-tetramethylethane-1,2-diamine (TEMED) (Sigma-Aldrich, Germany), ammonium persulfate (APS) (TH. Geyer Chemsolute, Germany), epichlorohydrin (ECH) (Sigma-Aldrich, Germany), ethanol (Sigma-Aldrich, Germany) and sodium hydroxide (Sigma-Aldrich, Germany) were used as received.

3.2 Cryogel Formation

Pullulan solutions of five different concentrations (26.32 g/L, 17.57 g/L, 13.16 g/L, 8.78 g/L, 4.39 g/L) were prepared by dissolving a given amount of pullulan in 25.0 mL 0.10 M NaOH solution. Aqueous solutions of ECH were prepared by adding either 0.6 mL, 1.1 mL, 1.6 mL, 2.1 mL ECH into 32 mL of distilled water. Then, HEMA (4.8 mL, 3.8 mL, 2.8 mL, 1.8 mL) was added into the pullulan solution by stirring. Mixing the pullulan solution containing HEMA with epichlorohydrin solution followed this step. Then, the solution was placed into an ice bath, APS (0.15 g, 0.30 g, 0.45 g, 0.60 g) was added and stirred, TEMED (0.1 mL) was added immediately after adding APS and the solution was stirred for a few seconds. The effect of EGDMA as a second crosslinker was tested by using 1.25 mL in some cases. The final solution was poured into a glass tube and placed in the freezer at -18 °C for 48 hours. At the end of the reaction period, cold ethanol solution at -18 °C was added on to the cryogel. Cryogels were left in ethanol for 24 h at room

temperature. After this period glasses were broken in a controllable manner to remove the cryogels out of the tubes. The sample was washed with distilled water and ethanol, and air dried to constant weight.

Above given typical procedure was repeated at different reaction conditions as given in Table 1.

Table 1: Cryogel formation parameters.

Sample ID	[Pullulan] (g/L)	[HEMA] (mol/L)	[APS] (mol/L)	[ECH] (mol/L)	[EGDMA] (mol/L)	T (°C)	Time (h)	Observation
1	26.32	0,7	0.006	0.14	-	-20	48	White gel with high viscosity
2	17.54	0,7	0.006	0.14	-	-20	48	White gel
3	13.16	0,7	0.006	0.14	-	-20	48	Colourless gel
4	8.78	0,7	0.006	0.14	-	-20	48	Colourless gel
5	4.39	0,7	0.006	0.14	-	-20	48	Colourless gel with low viscosity
6	17.54	0,7	0.006	0.14	-	-20	48	White gel
7	17.54	0,55	0.006	0.14	-	-20	48	White gel
8	17.54	0,40	0.006	0.14	-	-20	48	White gel
9	17.54	0,26	0.006	0.14	-	-20	48	White gel
10	17.54	0,7	0.006	0.25	-	-20	48	White gel
11	17.54	0,7	0.006	0.36	-	-20	48	White gel
12	17.54	0,7	0.006	0.47	-	-20	48	White gel
13	17.54	0,7	0.006	0.58	-	-20	48	White gel
14	26.32	0,7	0.012	0.14	-	-20	48	White gel
15	26.32	0,7	0.023	0.14	-	-20	48	White gel
16	26.32	0,7	0.035	0.14	-	-20	48	White gel
17	26.32	0,7	0.046	0.14	-	-20	48	White gel
18	25	0.62	0.0054	0.4	0.166	-18	48	White gel
19	25	0.48	0.0054	0.4	0.166	-18	48	White gel
20	25	0.35	0.0054	0.4	0.166	-18	48	White gel
21	25	0.23	0.0054	0.4	0.166	-18	48	White gel

3.3 FTIR-ATR Spectroscopy

Perkin Elmer Spectrum Two FTIR-ATR spectrometer was used to determine the chemical functionalities of samples. The samples were crushed into powder form for FTIR-ATR analysis.

3.4 Scanning Electron Microscopy

The surface morphology of the samples was investigated in the Central Laboratory of METU in Ankara by using Quanta 400F filed emission scanning microscope using Au-Pd coating.

3.5 Product Yield Description Equation

İt describes the polymerisation of the material used.

$$Yield\% = \frac{W_1}{W_T}$$

W_1 = Dried cryogel

W_T = Total weight

3.6 Gel Fraction Description Equation

Gel fraction is a term, which describes the amount of gel formed from the starting materials.

$$GF\% = \frac{W_1 - W_2}{W_1} X\ 100$$

W_1 = Dried cryogel

W_2 = Dried cryogel following to one day incubation in the water

3.7 Swelling Kinetics

SHIMADZU-TW423L model of analytical balance was used to record the increase in masses of cryogels at regular time intervals. As a swelling medium distilled water

was used. All the swelling results were given as % swelling and %swelling was calculated as follow;

$$\%\, Swelling = \frac{W_{swollen} - W_{dried}}{W_{dried}}\ X\ 100 \qquad (1)$$

3.8 Percent Loss of Weight of Cryogel Systems

The masses of dried cryogels were measured. Following to this, they were incubated in acidic (pH ≈1), basic (pH ≈11), and neutral (pH ≈ 7.4). Then, they were dried in oven at 40 °C for two days and the final masses of the cryogels were recorded. This was repeated for several time.

$$loss\ of\ weight\ \% = \frac{W_i - W_f}{W_i}\ X\ 100$$

W_i = Weight initial

W_f = Weight final

Chapter 4

RESULTS AND DISCUSSION

4.1 Pullulan-PolyHEMA Cryogel Formation

During pullulan-PolyHEMA cryogel formation, grafting of polyHEMA onto pullulan occurs via APS initiation. In addition to grafting reaction at low temperatures, pullulan undergoes crosslinking reaction with epichlorohydrin (ECH). Hydrogen bonding interactions between pullulan-pullulan, pullulan-polyHEMA, polyHEMA-polyHEMA chains also contribute to gel formation. Chemical crosslinking of pullulan with ECH, and hydrogen bonding interactions between pullulan-pullulan, pullulan-polyHEMA, polyHEMA-polyHEMA chains are shown in Figure 5, 6,7 and 8 respectively. Furthermore, the effect of EGDMA crosslinking on cryogel formation was tested. The crosslinking reaction between HEMA and EGDMA is shown in Figure 9. The product obtained is expected to be pullulan-ECH-*graft*-polyHEMA-EGDMA. A schematic representation of the ECH and EGDMA crosslinked pullulan/polyHEMA cryogel is given in Figure 10.

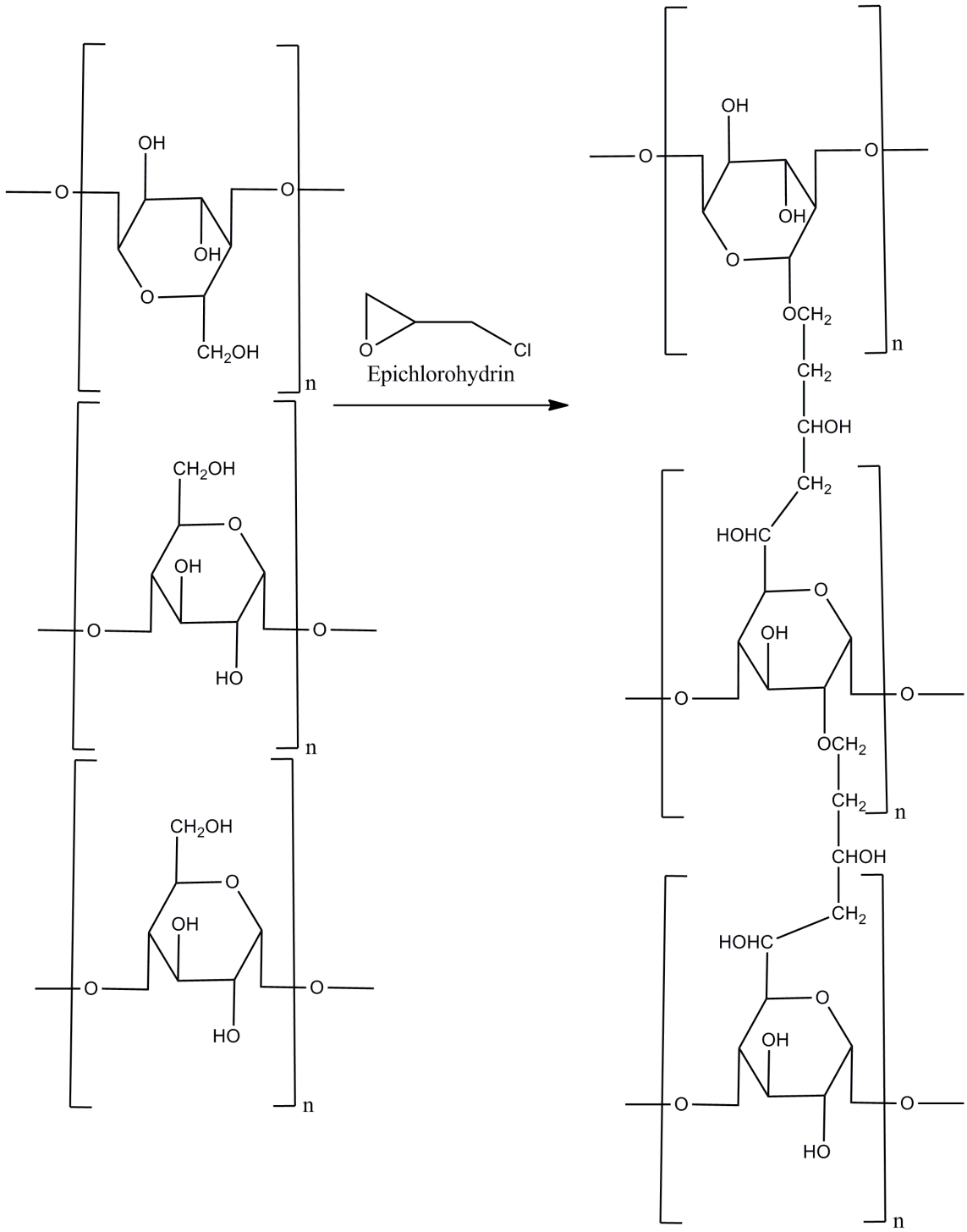

Figure 5:Pullulan crosslinked with epichlorohydrin.

Pullulan and polyHEMA undergo crosslinking reactions via the help of hydrogen bonds as shown in Figure 6 , Figure 7 and Figure 8.

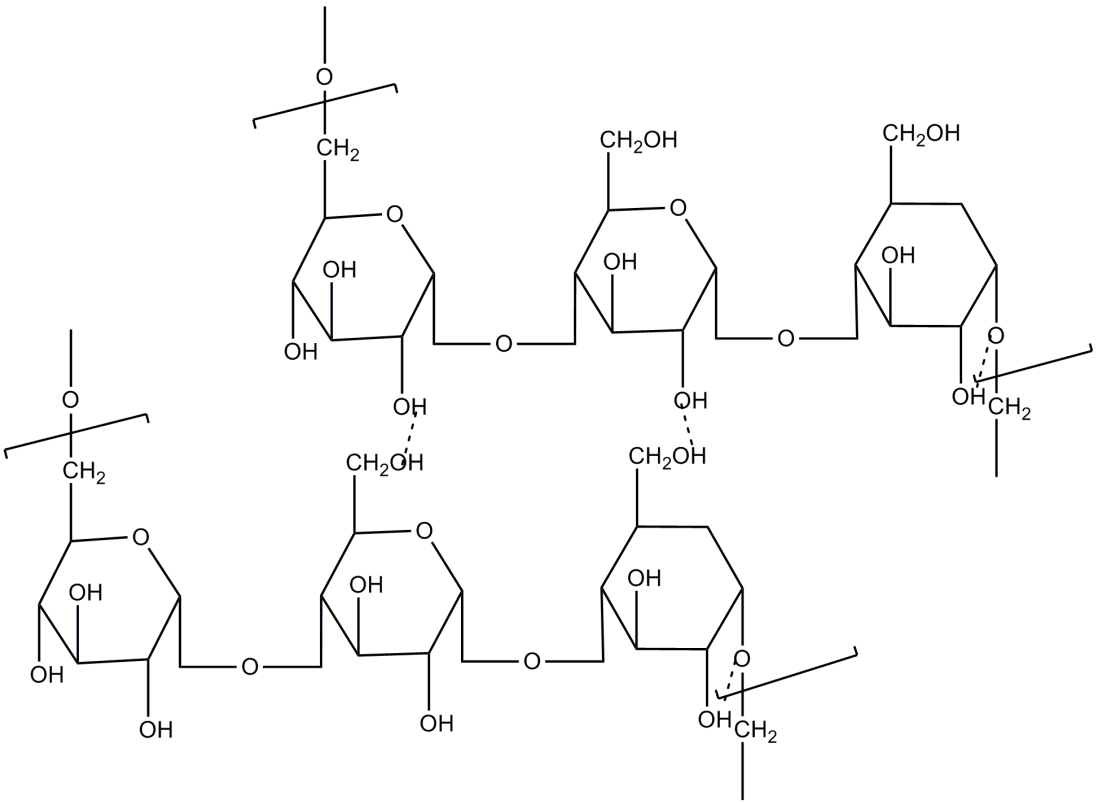

Figure 6: Pullulan-pullulan hydrogen bond interactions.

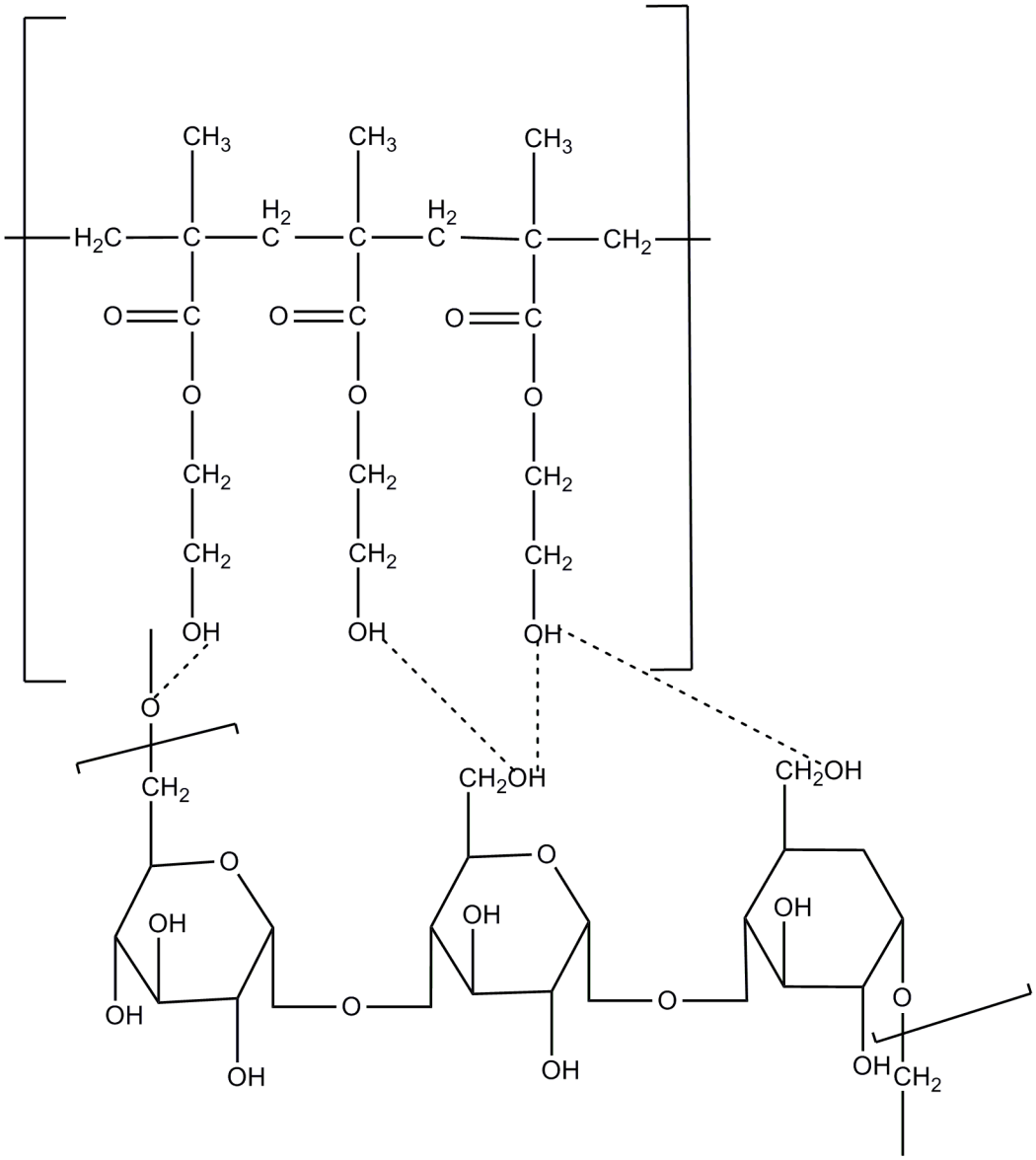

Figure 7: PolyHEMA- Pullulan hydrogen bond interactions.

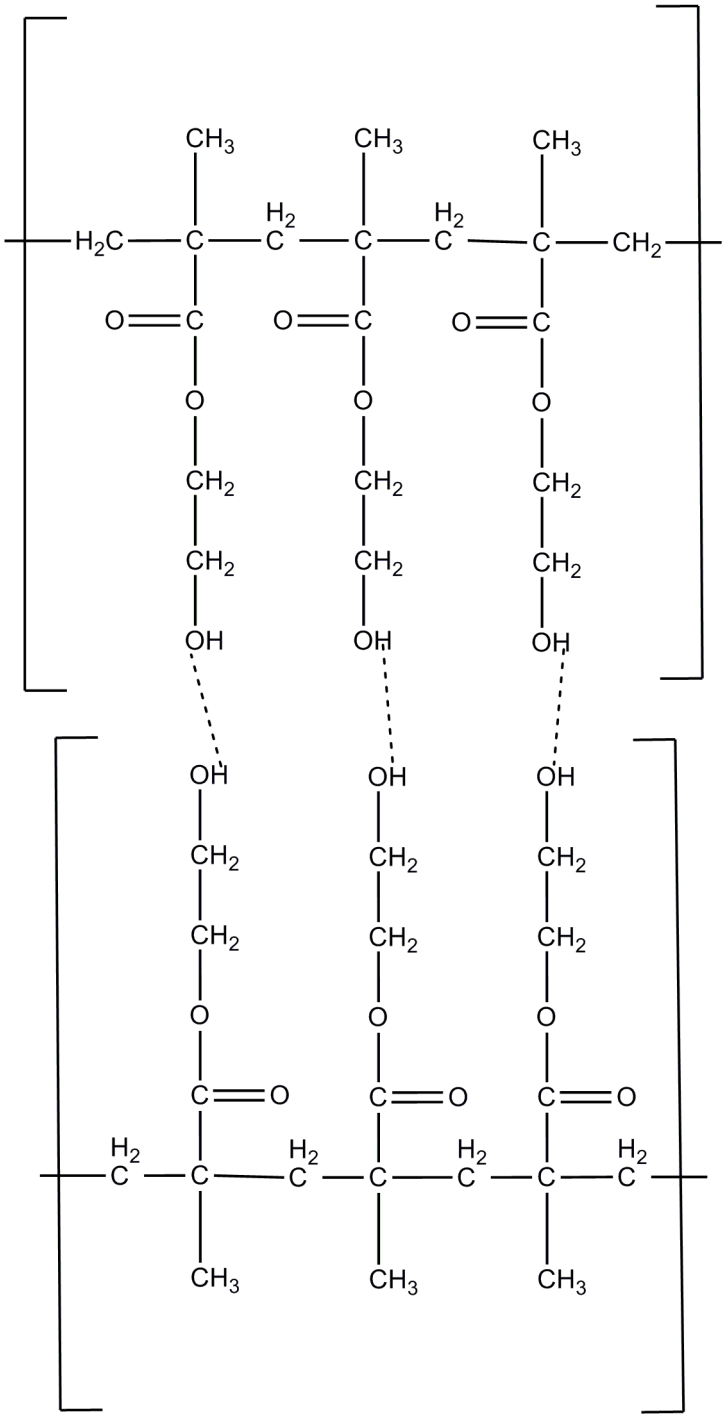

Figure 8: PolyHEMA-polyHEMA hydrogen bond interactions.

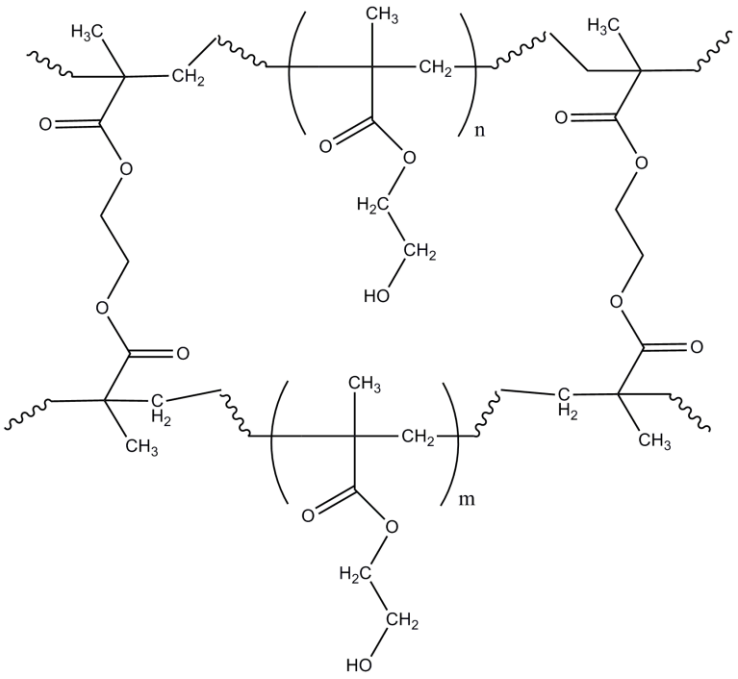

Figure 9: PolyHEMA cross-linked withethylene glycol dimethacrylate.

The effect of ECH concentration, pullulan concentration, HEMA and APS concentrations on the yield% and gel fraction% of the cryogels was studied. The reaction conditions and the results are summarized in Table 2.

4.2 Effect of ECH Concentration on Yield and Gel Fractions

The effect of ECH concentration on the yield % and gel fraction% is shown in Figure 11. Yield percentage increases from 16.9 % to 26.5 % on the average when ECH concentration changes between 0.01875 M - 0.04 M. The amount of ECH used, limits the yield values to 26.5 %. Increasing ECH concentration from 0.04 M to 0.08 M does not have a significant effect on the yield percentage value. Gel fraction increases from 75 % to 90% upon increasing ECH concentration to 0.04 M.

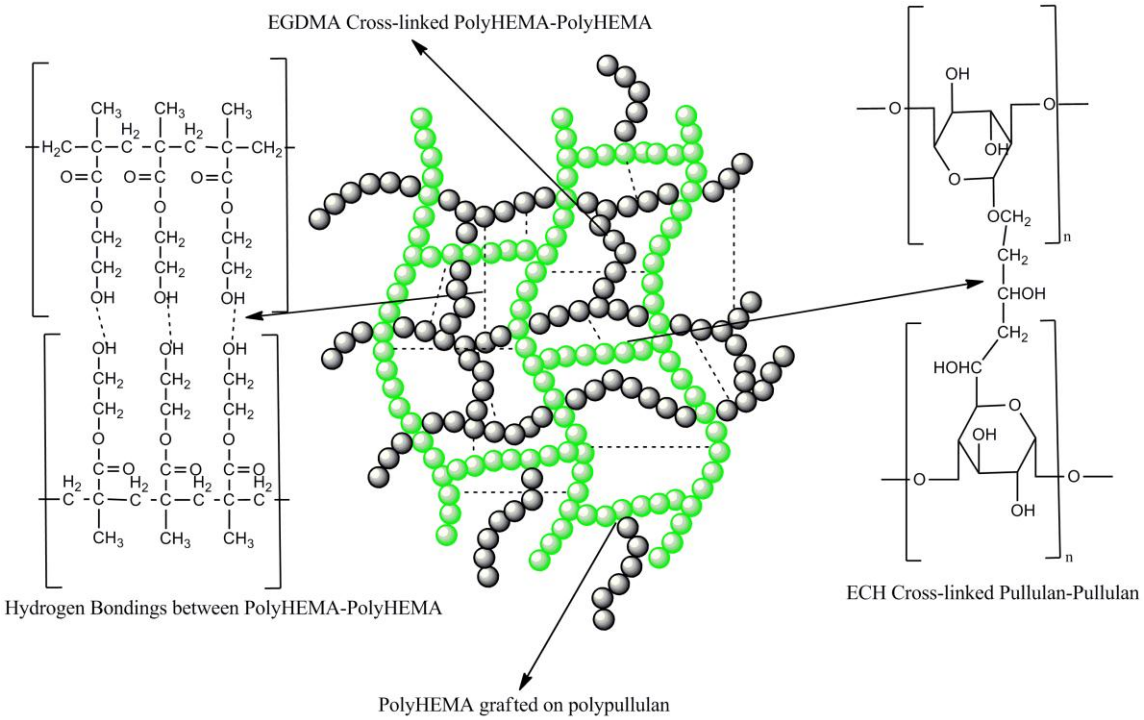

Figure 10: Schematic representative of the pullulan-polyHEMA cryogels.

Table 2: Yield and gel fraction table with other parameters.

Sample ID	[Pullulan] (g/L)	[HEMA] (mol/L)	[APS] (mol/L)	[ECH] (mol/L)	[EGDMA] (mol/L)	T (°C)	Time (h)	Observation	GF %	Yield%
1	26.32	0,7	0.006	0.14	-	-20	48	White gel with high viscosity	66.33	85.7
2	17.54	0,7	0.006	0.14	-	-20	48	White gel	94.01	33.1
3	13.16	0,7	0.006	0.14	-	-20	48	Colourless gel	78.24	16.9
4	8.78	0,7	0.006	0.14	-	-20	48	Colourless gel	92.3	14.3
5	4.39	0,7	0.006	0.14	-	-20	48	Colourless gel with low viscosity	91.13	11.6
6	17.54	0,7	0.006	0.14	-	-20	48	White gel	88.13	38.8
7	17.54	0,55	0.006	0.14	-	-20	48	White gel	84.03	42.2
8	17.54	0,40	0.006	0.14	-	-20	48	White gel	84.60	48.5
9	17.54	0,26	0.006	0.14	-	-20	48	White gel	87.97	64.5
10	17.54	0,7	0.006	0.25	-	-20	48	White gel	90.89	90.89
11	17.54	0,7	0.006	0.36	-	-20	48	White gel	91.53	91.53
12	17.54	0,7	0.006	0.47	-	-20	48	White gel	84.5	84.5
13	17.54	0,7	0.006	0.58	-	-20	48	White gel	90.2	90.2
14	26.32	0,7	0.012	0.14	-	-20	48	White gel	85.66	83.3
15	26.32	0,7	0.023	0.14	-	-20	48	White gel	80.39	70.9
16	26.32	0,7	0.035	0.14	-	-20	48	White gel	78	84.2
17	26.32	0,7	0.046	0.14	-	-20	48	White gel	68.53	55.9
18	25	0.62	0.0054	0.4	0.166	-20	48	White gel	82.5	82.5
19	25	0.48	0.0054	0.4	0.166	-20	48	White gel	81.2	81.2
20	25	0.35	0.0054	0.4	0.166	-20	48	White gel	82.7	82.7
21	25	0.23	0.0054	0.4	0.166	-20	48	White gel	78.6	78.6

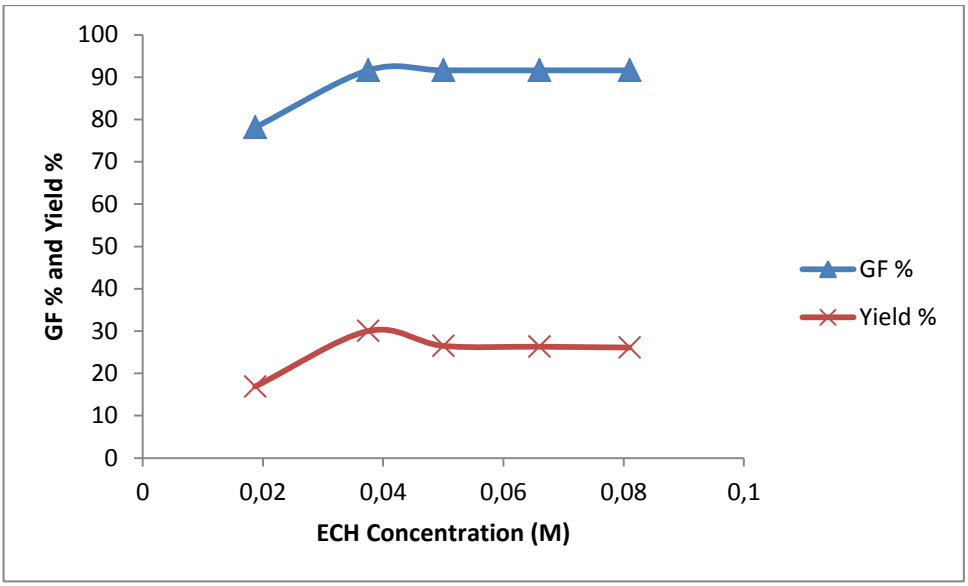

Figure 11: Effect of ECH concentration on yield and gel fraction.

4.3 Effect of APS Concentration on Yield and Gel Fraction

When APS concentration is increased from 0.012 M to 0.046 M, %yield and %gel fraction values are decreased. Gel fraction is decreased from 85.66% to 68.53%, meanwhile, yield is dropped from 83.3% to 55.9%. As the concentration of APS is increased, the radical concentration increases. Possibility of reactions between reactive ends of growing chain polymers is increased. Reactive ends of polymer chains react with each other to terminate the polymerization. Yield and gel fractions decrease as the concentration of APS in the cryogelation medium increase as shown in Figure 12.

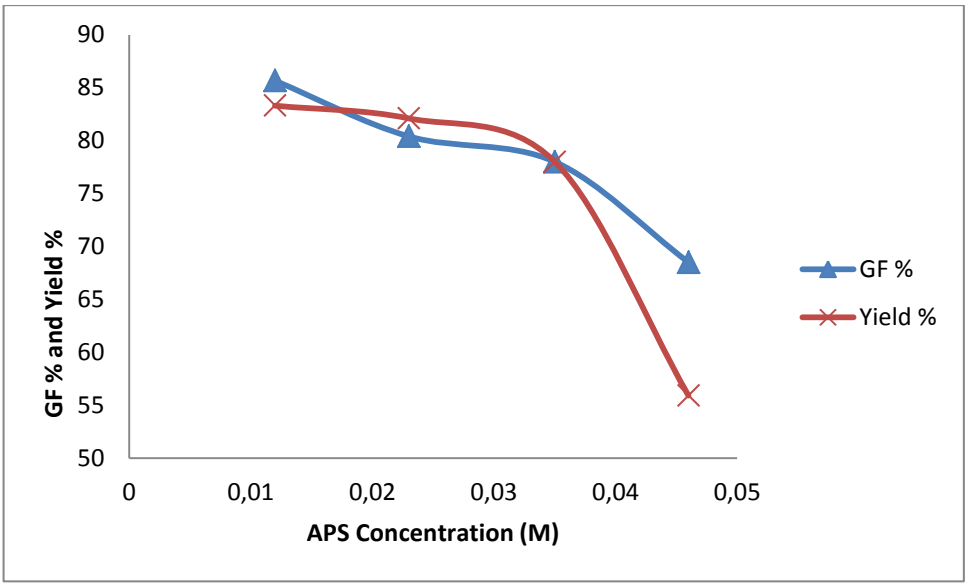

Figure 12: Effect of APS concentration on yield and gel fraction.

4.4 Effect of Pullulan Concentration on Yield and Gel Fraction

The effect of pullulan concentration on yield% and gel fraction% is shown in Figure 13.. As the amount of pullulan increases in the nonfrozen microphases, ECH crosslinked pullulans form, as ECH has affinity to crosslink the pullulan polymer instead of HEMA. Therfore, yield% increases with increasing pullulan concentration. Interactions between Pullulan-pullulan, pullulan-HEMA, HEMA-HEMA polymers are secondary interactions such as hydrogen bondings. Therefore, pullulan chains, which are not chemically crosslinked are dissolved in water. This leads to decrease in gel fraction due to loss of polymer dissolution in water.

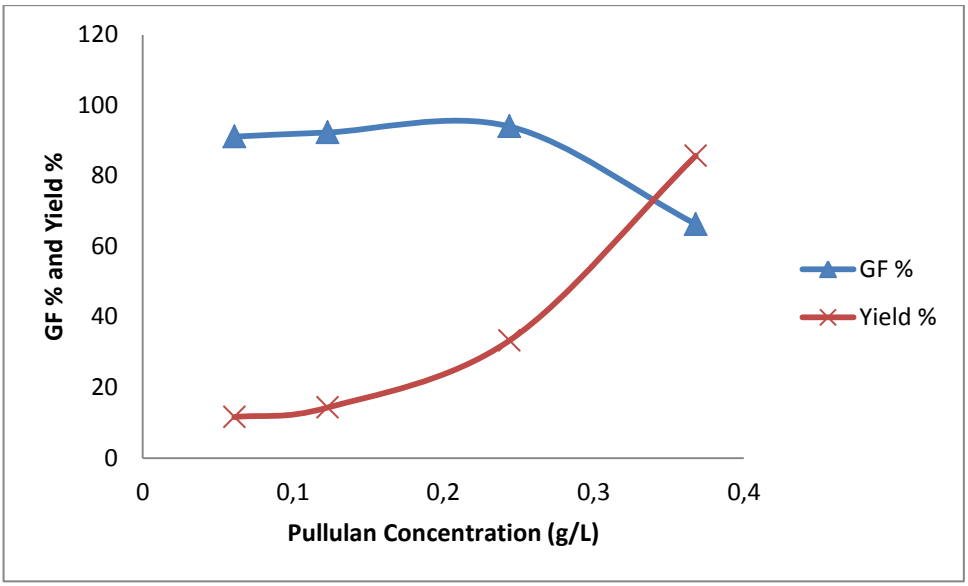

Figure 13: Effect of pullulan concentration on yield and gel fraction.

4.5 Effect of HEMA Concentration on Yield and Gel Fraction

The effect of HEMA concentration on yield% and gel fraction% is given in Figure 14. As the concentration of HEMA is increased, yield decreases. This should be due to the fact that excess HEMA remains in the unfrozen liquid microphases, and does not contribute to product formation as pullulan chains become crosslinked with ECH. The network formed hinders diffusion of HEMA molecules into the gel structure under cryogelation conditions. There is only a small increase in the % gel fraction. indicating that the amount of HEMA does not affect gel content. The predominating factor is the amount of pullulan in the medium.

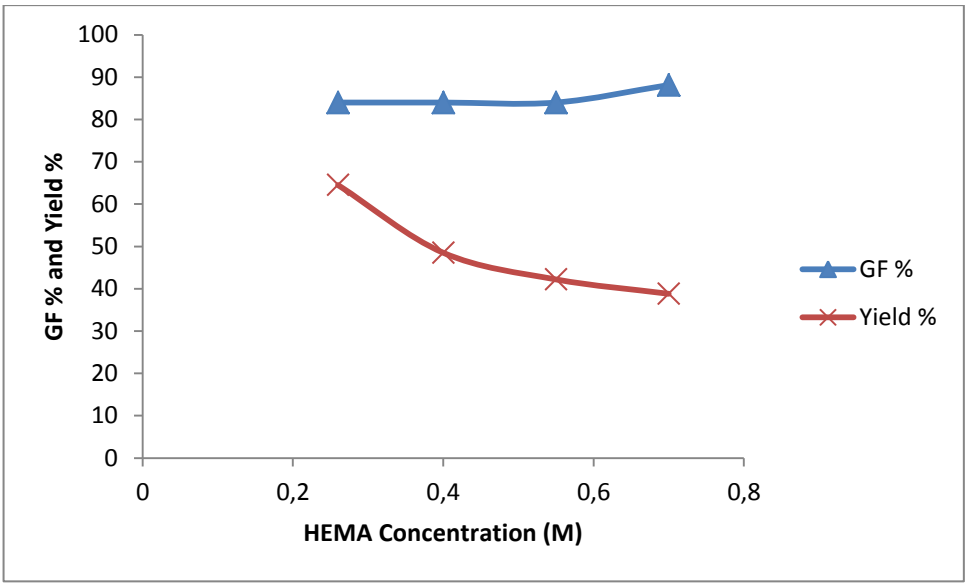

Figure 14: Effect of HEMA concentration on yield and gel fraction.

4.6 Effect of HEMA Concentration with Presence of EGDMA on Yield and Gel Fraction

The effect of HEMA concentration on the yield% and gel fraction% is shown in Figure15. It can be followed from Figure 15 that crosslinking of HEMA with EGDMA contributes to gel formation considerably. In contrast to what is observed in Figure 14, increasing HEMA concentration results in more yield. This should be due to crosslinking of polyHEMA with EGDMA. Hence, even though not all of HEMA may be grafted on ECH crosslinked pullulan, EGDMA crosslinked polyHEMA chains are formed and exist together with crosslinked and grafted pullulan chains.

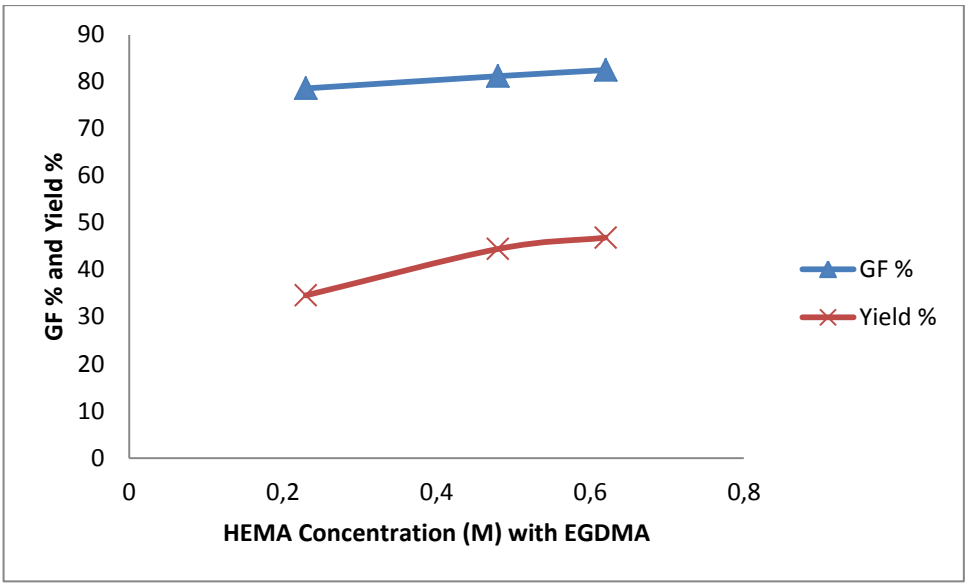

Figure 15: Effect of HEMA Concentration with presence of EGDMA on yield and gel fraction.

4.7 FT-IR Analysis

FTIR spectrum of ECH is given in Figure 16. Peaks at 960.99 cm^{-1} and 926.16 cm^{-1} represent deformation of C-C single bond of epoxide group of epichlorohydrin. Very strong band at 3004.01 cm^{-1} indicates asymmetric stretching of methylene groups. Band centered at 1266.60 cm^{-1} represents vibrational stretchings of C-C single bonds. Peak at 1136.56 cm^{-1} was observed due to the presence of vibrational stretching of C-O bond of ester. Very strong band at 721.39 cm^{-1} represents C-Cl stretching vibrations. Usually halogen containing compounds have significant peak between the wavelengths of 800 and 600 cm^{-1}.

FTIR spectrum of EGDMA is given in Figure 17. Peaks at 1717.18 cm^{-1} and 1145.03 cm^{-1} represent C=O double bond stretching and C-O single bond stretching, respectively. Presence of these two peaks were the main characteristic features of EGDMA. C=C double bonds were indicated by the presence of peak at 1637.62 cm^{-1} due to the vibrational stretchings.

FTIR spectrum of HEMA as given in Figure 18; Peaks at 3425.11 cm^{-1} and 2957.56 cm^{-1} represent vibrational stretchings from hydroxyl groups of HEMA and $–CH_2$ groups, respectively. Peaks at 1636.90 cm^{-1}, 943.04 cm^{-1}, and 815.04 cm^{-1} represent the presence of C=C double bond in the structure of HEMA. C=O double bonds cause vibrational stretchings and this was detected at 1715.95 cm^{-1}. On the other hand, peak at 1160.89 cm^{-1} represents vibrational stretching due to the presence of C-O single bond.

FTIR spectrum of pullulan as given in Figure 19; Peaks at 3307.49 cm^{-1} and 2922.19 cm^{-1} represent presence of O-H bonds and sp^3 $–CH_2$ groups, respectively. Peak at 1644.86 cm^{-1} indicates the presence of O-C-O bond in pullulan moiety. C-OH bends and C-O-C stretching were observed at 1356.12 cm^{-1} and 1148.49 cm^{-1} , respectively. C_6-OH moiety give rise to a very sharp peak at 1003.33 cm^{-1}.

FTIR results of sample 18 as given in Figure 20; They were pullulan-HEMA cryogels in the presence of 0.4 M ECH and 0.166 M EGDMA. Peaks at around 1714 cm^{-1} and 1153 cm^{-1} represent C=O double bond and C-O single bond stretching from EGDMA crosslinker, respectively. Peaks at around 1020 cm^{-1} and 1075 cm^{-1} were indicating the presence of pullulan structure due to the vibrations of C_6-OH groups. Peak at around 1250 cm^{-1} was from HEMA and it represents the presence of C-O bond vibrations of ester group. In addition, peaks at around 1713 and 1153 cm^{-1} also indicate the presence of HEMA due to C=O double bond and C-O single bond vibrations, respectively.

FTIR spectrum of sample 8 and sample 1 as given in Figures 21 and 22, respectively; Sample 8 cryogel was formed in the presence of 0.14 M ECH and 0.244 M pullulan.

Sample 1 was pullulan-HEMA cryogels formed in the presence of 0.14 M ECH and 0.7 M HEMA.

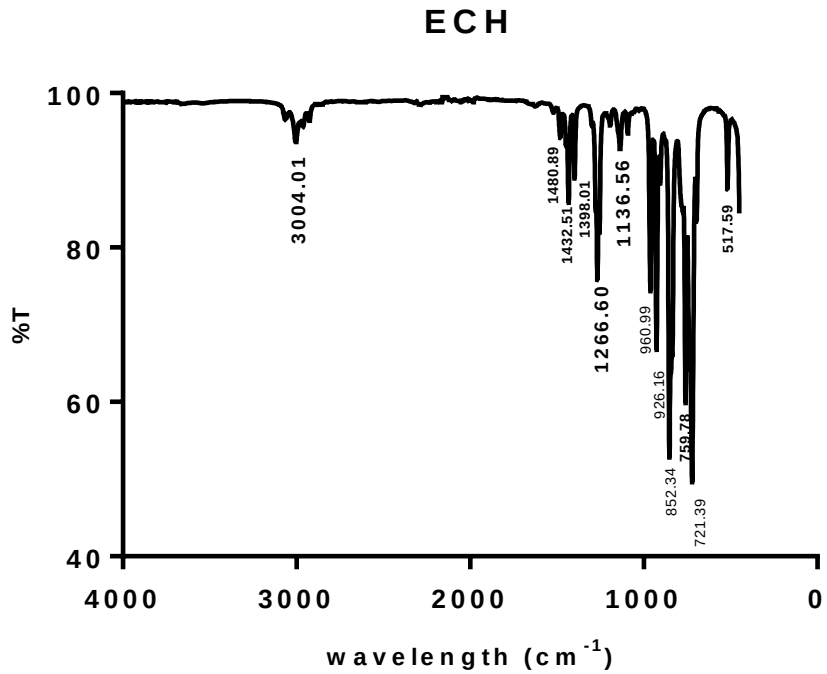

Figure 16: FT-IR spectrum of ECH.

It was observed that changing HEMA concentration or varying pullulan concentrations does not affect a change in the FTIR spectra. FTIR results were similar with each other. Peaks at around 1713 cm^{-1} and 1153 cm^{-1} represent the presence of C=O double bonds and C-O single bonds vibrational stretching from HEMA. Peaks at around 1075 cm^{-1} and 1020 cm^{-1} represent the vibrations of C_6-OH moieties of pullulan structure.

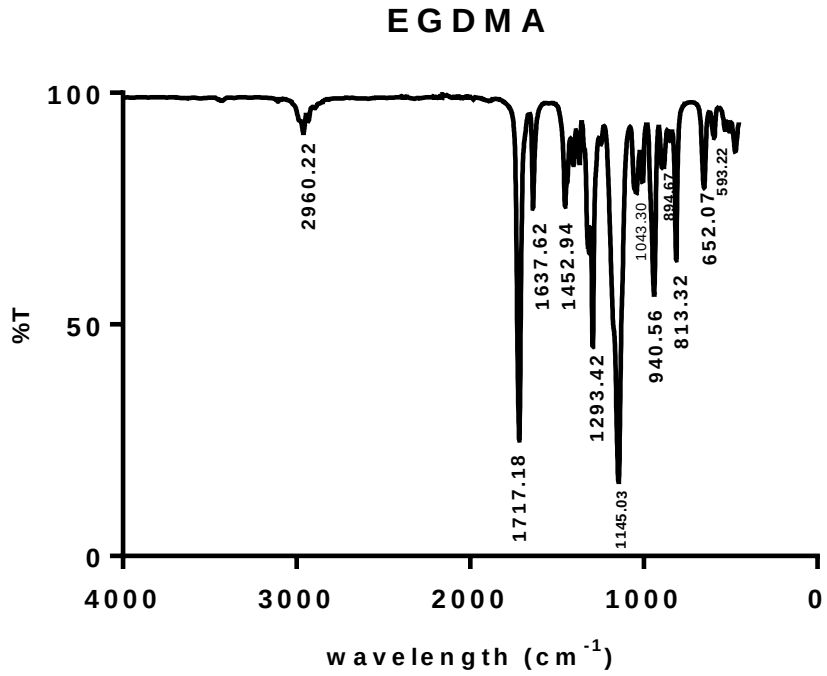

Figure 17: FT-IR spectrum of EGDMA.

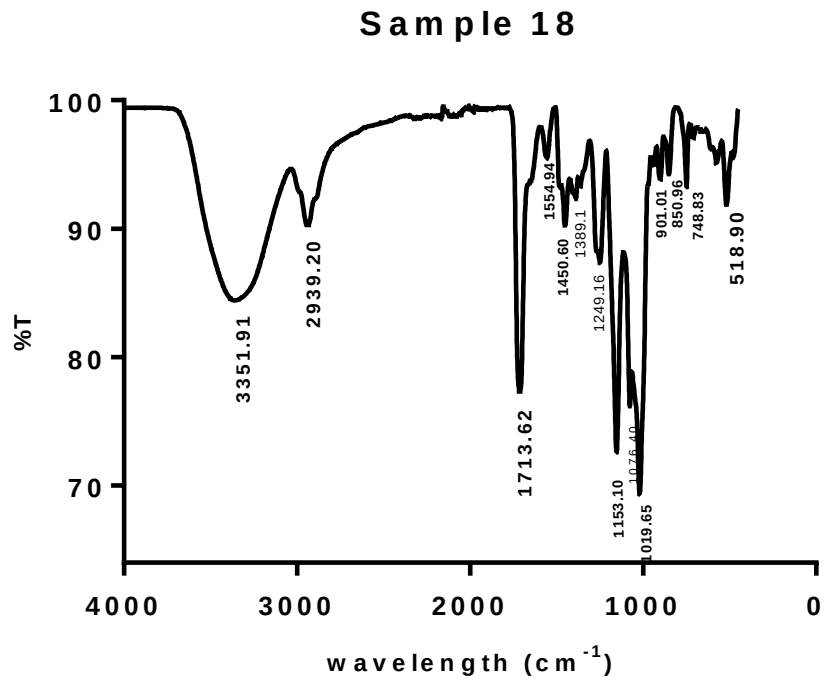

Figure 18: FT-IR spectrum of sample 18.

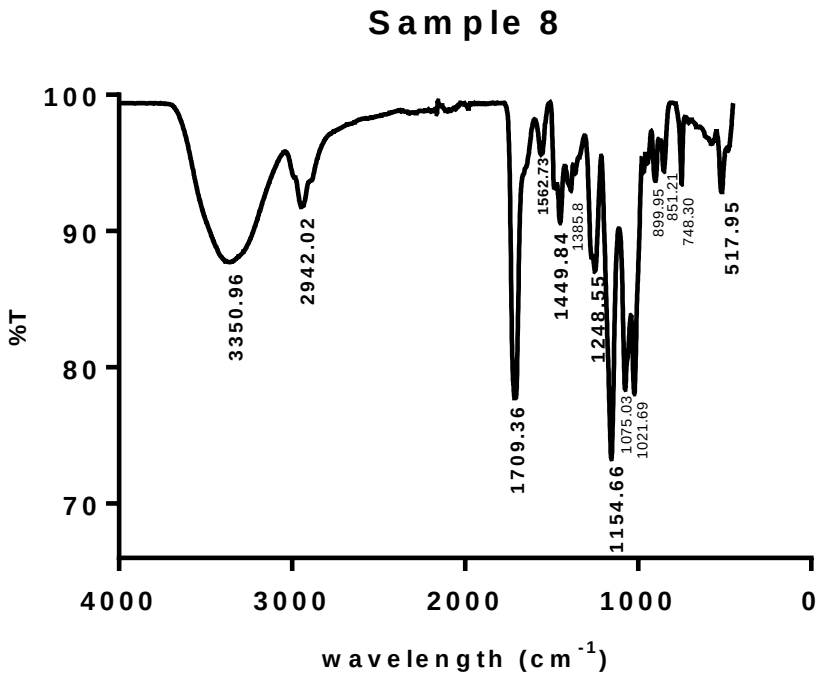

Figure 19: FT-IR spectrum of sample 8.

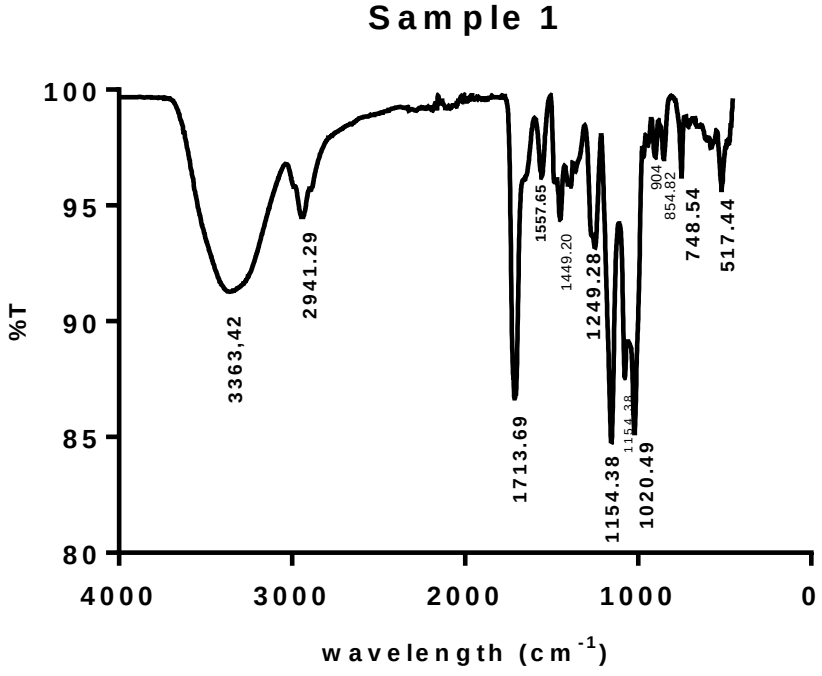

Figure 20: FT-IR spectrum of sample 1.

4.8 Swelling Results

The swelling ability of a cryogel is directly related to its water permeability and uptake which depends on its hydrophilicity. Presence of porous structure within a cryogel leads to the importance of swelling kinetics.

4.8.1 Effect of Varying Concentrations of PolyHEMA on Swelling

Swelling ratio determination for Pullulan-PolyHEMA cryogel was initially tested for the effect of HEMA concentration on swelling % . Increased concentration values of HEMA from 0.26 M to 0.7 M results in decrease of swelling % from 940 to 824. As discussed above in section 4.5, increasing HEMA concentration gives rise to less product yield indicating that tighter network forms between pullulan chains by ECH crosslinking, which hinders diffusion of HEMA monomer into network. Swelling results support this proposal as equilibrium swelling values decrease with increasing HEMA concentration.

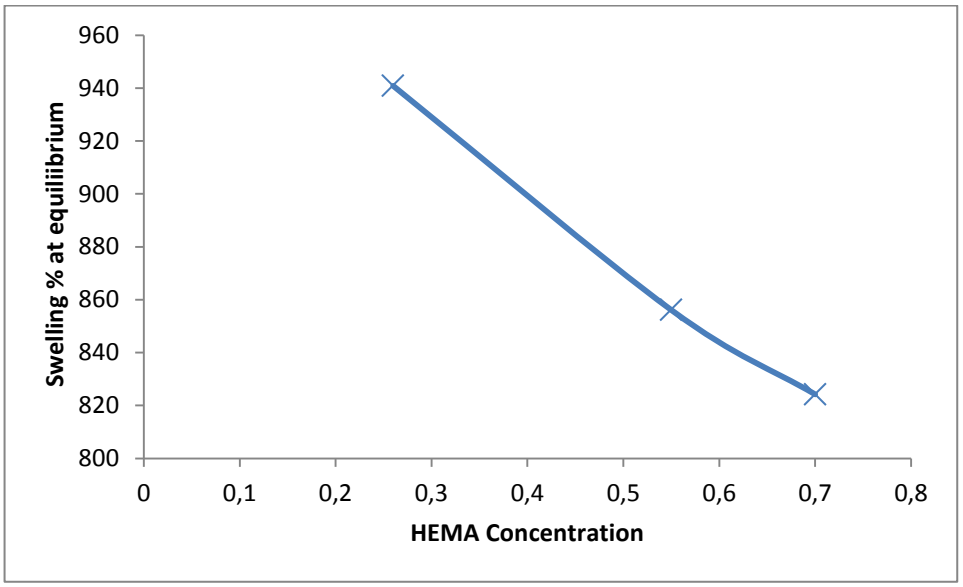

Figure 21: Effect of varying concentrations of PolyHEMA on swelling.

4.8.2 Effect of Varying Concentrations of Pullulan on Swelling

In addition to HEMA concentration, effect of Pullulan concentration on swelling % was also tested. Swelling % was decreased from 949 to 599 upon increasing concentration of Pullulan from 0.066 M to 0.368 M. Presence of chemical crosslinker ECH and its reaction with Pullulan as well as hydrogen bond formation between Pullulan-Pullulan and Pullulan-HEMA leads to the observed decrease in swelling %. This decrease in swelling % is similar and enhanced to results observed in HEMA concentration. Enhancement indicates that the effect of Pullulan on swelling % is greater than the effect of HEMA concentration as shown in Figure 24.

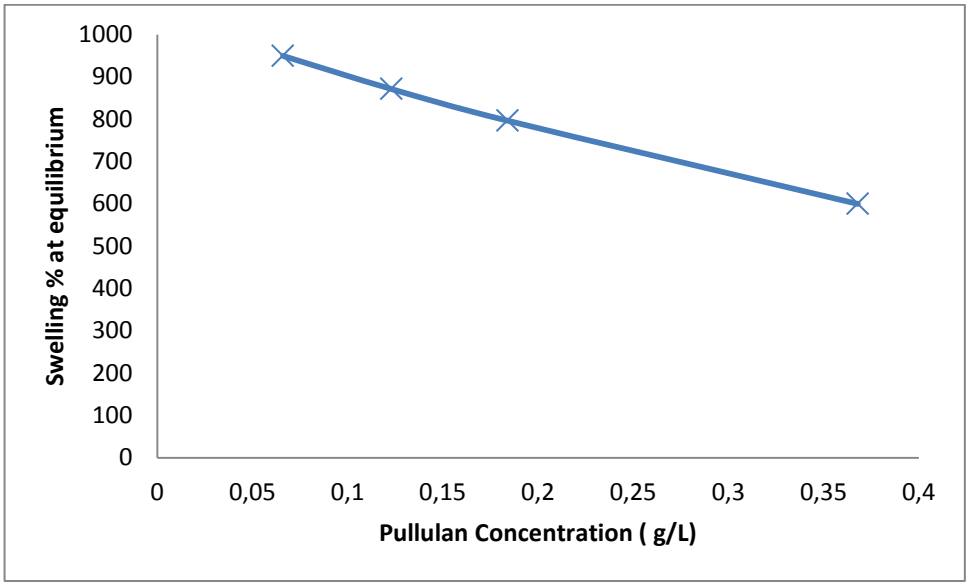

Figure 22: Effect of varying concentrations of Pullulan on swelling.

4.8.3 Effect of varying concenrations of HEMA with EGDMA on swelling

When the concentration of HEMA decreased from 0.62 M to 0.48 M, percent swelling decreased from 4889 % to 3040 % as given in Figure 25. As the HEMA concentration decreases, there is more probability for crosslinking with the EGDMA molecules available in the medium. Therefore, degree of crosslinking increases decreasing the swelling capacity. Furthermore, SEM pictures reveal that the cryogels

with EGDMA hav higher porosity than the others. This could be the reason for much higher swelling capacity values of the cryogels containing EGDMA than the ones without EGDMA.

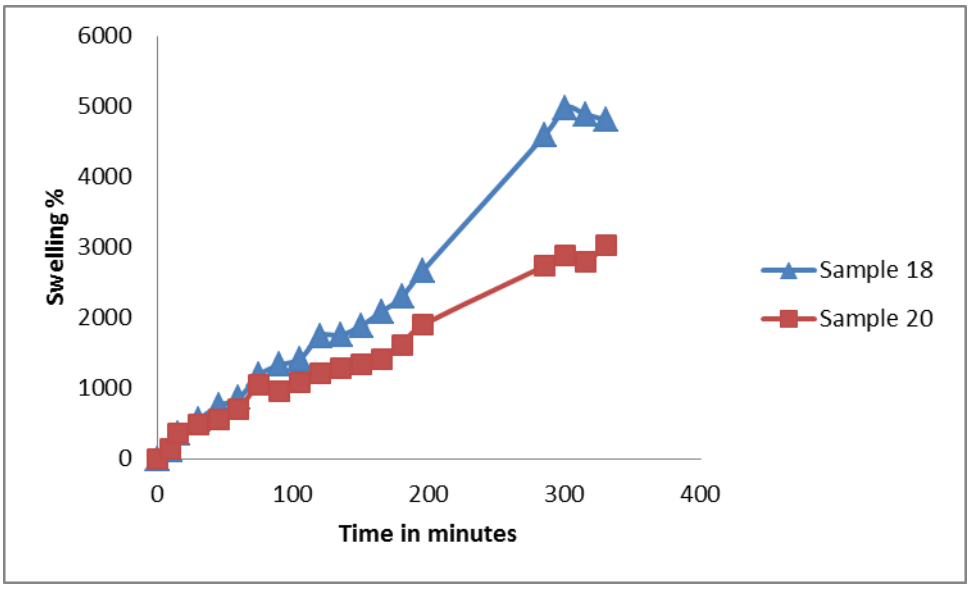

Figure 23: Effect of varying concentrations of PHEMA with EGDMA on swelling

4.8.4 Effect of pH on Swelling of Cryogels

The best swelling percents were observed with neutral and alkaline conditions. Cryogel systems showed the lowest swelling in acidic medium. Due to the aciditiy of the medium, the alcohol groups on pullulan and HEMA units may become protonated. The partial negative charge on these units is accompanied by the counter ion, chloride. The available empty space within the structure becomes relatively limited. Hence, diffusion of water molecules into the network structure is hindered, decreasing the swelling capacity.

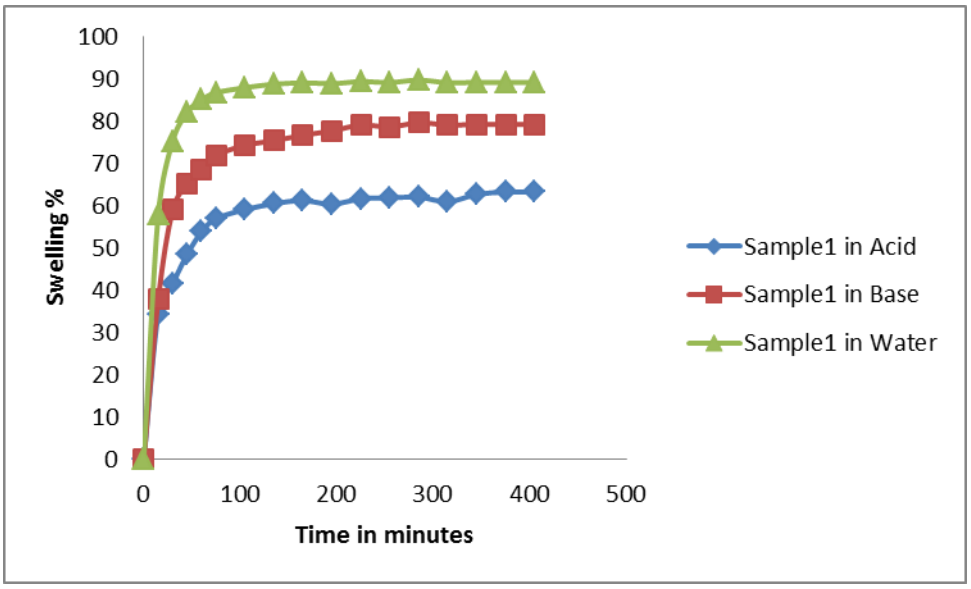

Figure 24: Percent Swelling of Sample 1

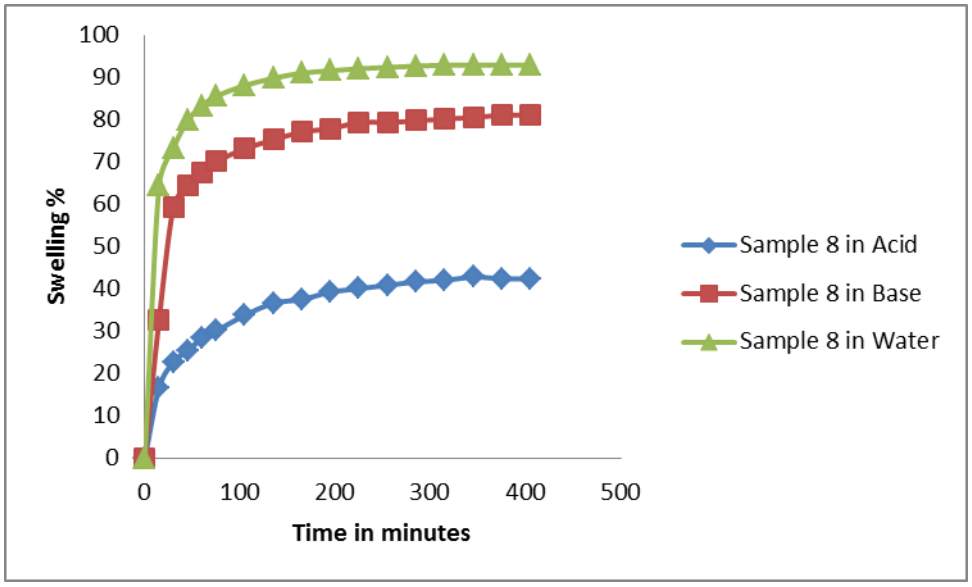

Figure 25: Percent Swelling of Sample 8

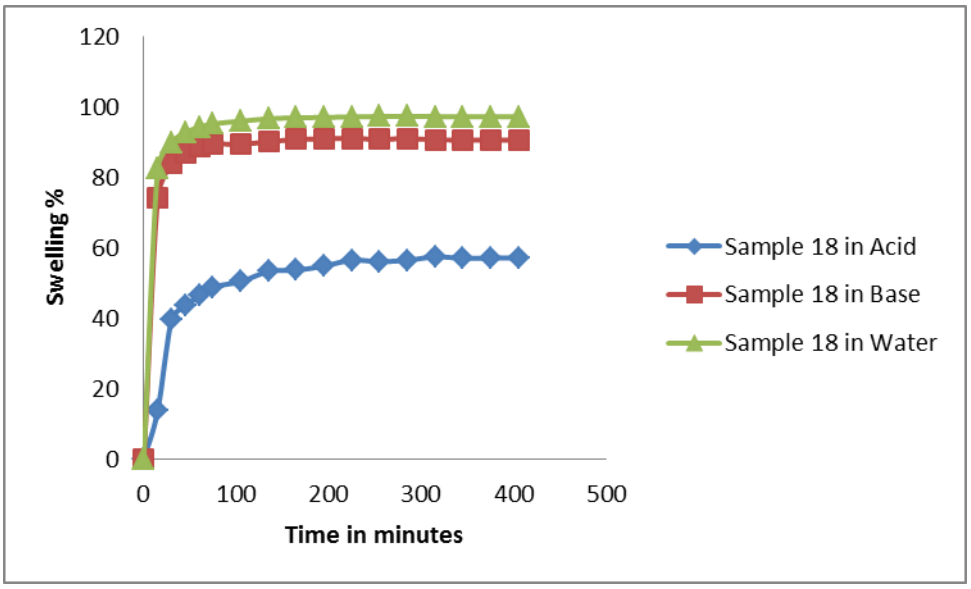

Figure 26: Percent Swelling of Sample 18

4.9 Percent Loss of Weight of Cryogel Systems

Weight loss in cryogels in acidic, basic and neutral conditions, is shown in Figure 29, 30 and 31 for samples 1, 8 and 18 respectively. Pullulan-ECH-*graft*-polyHEMA cryogels show the highest percent loss in weight in acidic medium. Average percent loss is around 9.50 %. This is because of the solution degradation of pullulan. Pullulan can degrade in aqueous media. In fact, it is more vulnerable to degradation in acidic medium. Due to the harsh acidic environment (pH=1), glycosidic linkages between pullulan repeating units could be subjected to damage. This would lead to damage on crosslinked chains. Pullulan-ECH-*graft*-polyHEMA-EGDMA cryogels showed the highest weight loss in water and the percent loss in weight was around 34.95%. This is because of the high percent swelling values in water. High swelling means that they can hold large amount of water molecules and in time this would cause the mechanical strength of the cryogels to be weaker than the normal conditions leading to loss of integrity.

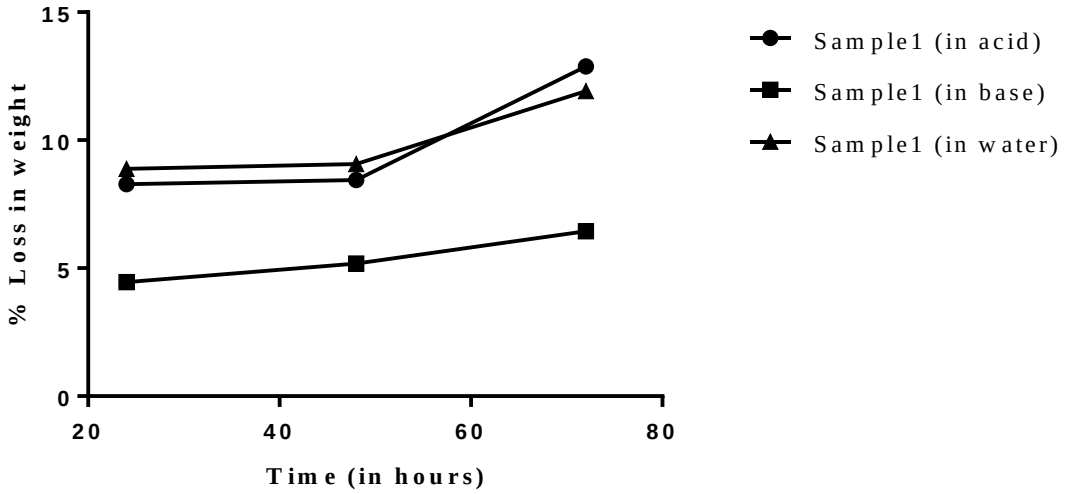

Figure 27: Percent loss in weight for sample 1.

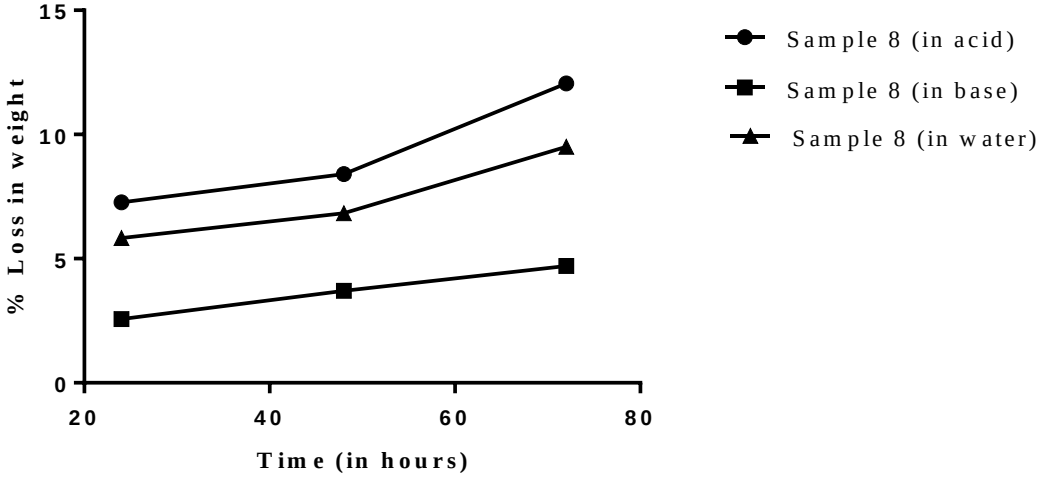

Figure 28 : Percent loss in weight for sample 8.

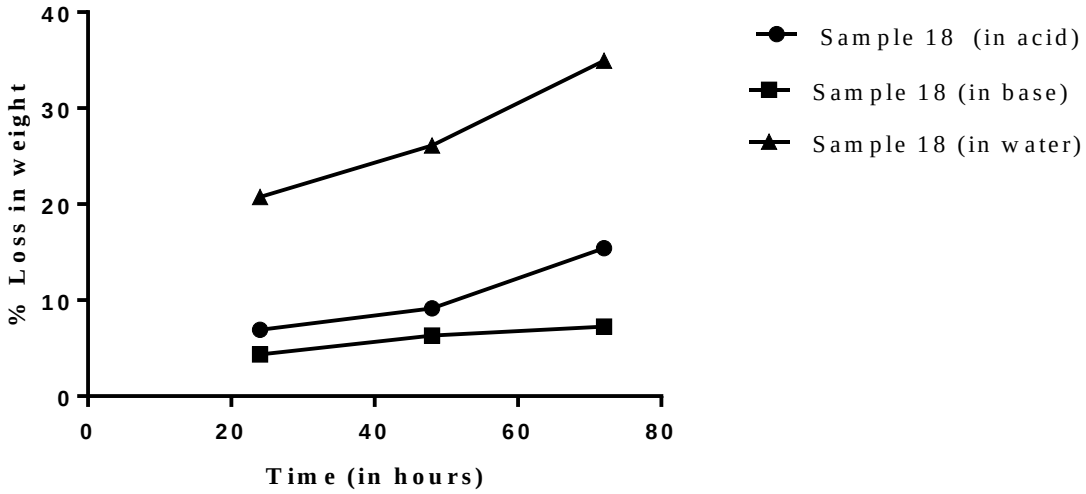

Figure 29: Percent loss in weight for sample 18.

4.10 Scanning Electron Microscopy (SEM) Results

SEM pictures of samples 1,3,6,8,18 and 20 are given in Figure 32,33 and 34 respectively. All samples exhibit porosity to a given degree due to the cryogelation conditions.

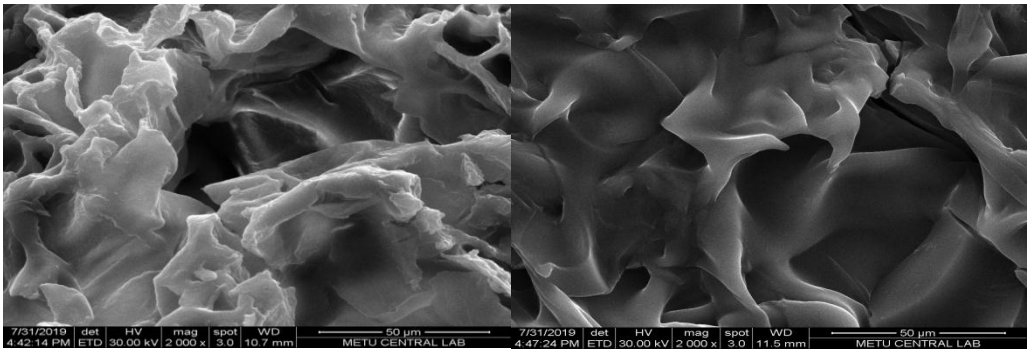

Figure 30: SEM images of sample 18(on the left) and sample 20(on the right).

In Figure 32 sem images of sample 18 and sample 20 are shown. It can be followed from Table 1 and Table 2 sample 20 has been synthesize using a higher concentration of HEMA then sample 18.As the HEMA concentration icreases, degree of porosity per unit area also inreases as can be observed in Figure 32.The main reason of this higher porosity is the concentration of starting polymer ;HEMA.It has been explained using Figure 14 that icreasing HEMA concentration results in lower gel fractions causing lower crosslinker density and looser network formation.Hence ,degree of porosity increases. In contrast ,compared to other types of cryogels EGDMA containing cryogels showed higher degree of porosity.

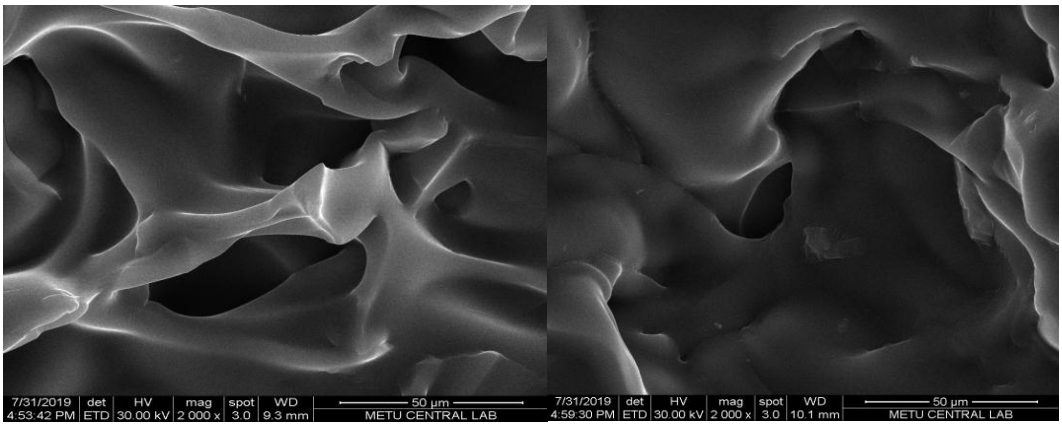

Figure 31: SEM images of sample 6 (on the left) and sample8 (on the right).

Figure 33 shows SEM images of sample 6 and sample 8 that were synthesize without EGDMA.When Figure 32 and Figure 33 are compared it can be concluded presence of EGDMA in the medium causes higher and more uniform porosity.

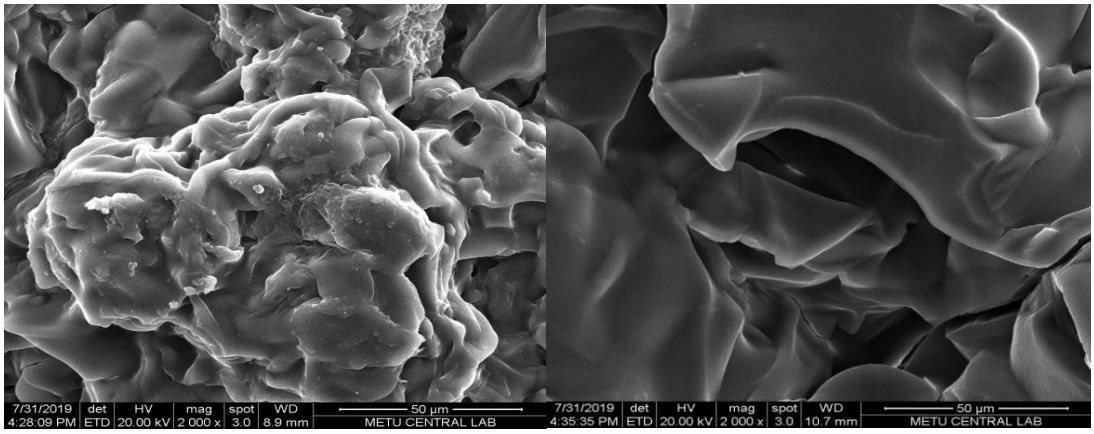

Figure 32: SEM images of sample 1 (on the left) and sample 3 (on the right).

When the SEM image of sample 1 is ccompared to that of sample3, it can be observed that sample 3 which contains less amount of pullulan has a smoother surface than sample 1 .When Figure 32 and Figure 34 are compared again it can be concluded that presence of EGDMA is critical in achieving porosity .

Chapter 5

CONCLUSION

In this thesis, synthesis and characterization of cryogel systems from varying concentrations of starting polymers or monomers were presented. The significant points of thesis thesis are summarized below.

Pullulan-ECH-*graft*-polyHEMA and pullulan-ECH-*graft*-polyHEMA-EGDMA cryogel systems can be synthesized by using free radical polymerization in the presence of ECH crosslinker at - 22°C within 48 hours.

For each of cryogel systems gel fractions were calculated. It was found that as the pullulan concentration increased, gel fraction was decreased. In contrast, as the HEMA concentration was decreased, gel fractions were not affected significantly. Concentration of ECH act like limiting reagent for the gel fraction. In addition, as the APS concentration increased, gel fraction was decreased.

*FTIR analysis gave data on the presence of pullulan and HEMA in the cryogel structure, and on the achievement of crosslinking by ECH .

* Due to the presence of hydrophilic groups in the structures of pullulan-ECH-*graft*-polyHEMA and pullulan-ECH-*graft*-polyHEMA-EGDMA cryogel systems, they showed high degree of swelling in water. Pullulan-ECH-*graft*-polyHEMA-EGDMA

cryogel systems can hold high amount of water molecules and this lead to decrease in the mechanical stability of the gels, in time.

SEM analysis revealed macroporous gel structure.

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