Cationic Pullulan via Poly(N-vinylimidazole) Grafting

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ABSTRACT

In this thesis, the possibility of grafting poly N-Vinylimidazole (PNVI) onto pullulan was investigated under homogenous and heterogenous conditions using potassium persulphate (KPS) and cerium(IV) ammonium nitrate (CAN) as redox initiators. It was found out that grafting of PNVI onto pullulan was succesful under heterogeneous conditions using any one of the initiators, CAN or KPS. Pullulan-*graft*-PNVI was obtained using 0.1000 g pullulan, 10 mL NVI , 0.5 g CAN at 60 °C for one hour in 100 mL toluene under nitrogen atmosphere with a grafting yield of 103%. Another pullulan-*graft*-PNVI sample was obtained under similar conditions using 0.5 g KPS with 162% grafting yield. Aqueous homogeneous reaction medium was not suitable for PNVI grafting onto pullulan.

Keywords: pullulan, graft copolymer, redox initiation, PNVI

ÖZ

Bu çalışmada poli(N-vinilimidazol)'un (PNVI) pululan üzerine aşılanma koşulları araştırılmıştır. Aşılanma reaksiyonu homojen ve heterojen koşullarda potasyum per sülfat (KPS) ve seryum (IV) amonyum nitrat (CAN) redoks başlatıcı kullanılarak çalışılmıştır. Aşılanma reaksiyonunun heterojen ortamda hem KPS hem de CAN başlatıcı ile gerçekleştiği saptanmıştır. Örneğin 0.5 g CAN başlatıcı ile 100 mL tolüen içinde ve azot atmosferinde, 60 °C sıcaklıkta 0.100 g pululan ve 10 mL NVI kullanılarak %103 aşılanma verimi elde edilmiştir. Benzer koşullarda 0.5 g KPS başlatıcı ile ise %162 aşılanma verimi ile ürün elde edilmiştir. Sulu homojen ortamın ise pululan üzerine aşılanma reaksiyonu için uygun bir ortam olmadığı tesbit edilmiştir.

Anahtar kelimeler: pululan, PNVI, redoks başlatıcı, aşı kopolimer

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Chapter 1

INTRODUCTION

Pullulan is a polysaccharide produced by *Aureobasidium pullulans* (*A. pullulans*). It is a linear polymer made up of maltotriose units linked by α-(1→6)-linkages. The polymer is freely soluble in water. It is a nontoxic, edible polymer widely used in the food industry, and as excipient in cosmetics and pharmaceutical formulations. As a neutral polysaccharide it has a potential to be applied as a blood-plasma substitute similar to dextran (Shingel, 2004).

Chemical modification of pullulan is possible via functional –OH groups available on the polymer backbone. Pullulan was modified by carboxymethylation, sulfation, chloromethylation . Cholesteryl group was substituted to introduce hydrophobicity to this hydrophilic polymer. The derivatization efforts were mainly aimed at improving blood anticoagulant properties of pullulan (Shingel, 2004).

Studies on modification of pullulan via grafting of synthetic polymers are rather rare. Two examples that can be mentioned are grafting of carboxy terminated PEG onto pullulan via an esterification reaction (Jiao,2004) and grafting of PMA using ceric ammonium nitrate as redox initiator (Wu, 2009).

The aim of this thesis is to synthesize poly (N-vinylimidazole) (PNVI) grafted pullulans. The importance of PNVI grafting is that hybridization of pullulan and PNVI will result in modified pullulan with cationic charge.

Since PNVI homopolymer is water soluble itself, it is expected that water soluble pullulan-*graft*-PNVI will be obtained. Among polysaccharides only chitosan bears inherent cationic charge due to the amine -NH_2 group present on the polymer repeat unit. The versatility of chitosan in biomedical applications is very well known (Yilmaz, 2006). The disadvantage of chitosan is that it is only soluble in aqueous acid solutions. It was established by experience in our lab is that polymer grafted chitosans are usually insoluble or partly soluble in aqueous media (Caner, 2007). Hence, pullulan-*graft*-PNVI has a potential to find a place in biomedical applications where water solubility is critical. For example, nontoxic, water soluble gene carriers with cationic charge strong enough to bind DNA effectively via complex formation, and weak enough to release DNA in the nucleus of the cell is of great interest. Furthermore, water soluble polymers with antibacterial activity are also needed.

1.1 Pullulan

Pullulan is a linear fungal polysaccharide made up of maltotriose units, linked by α-1,4-linked glucose molecules, 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). This organism is known as black yeast, and is found in soil, wastewater and the surface of synthetic materials (Shingel, 2004). The chemical structure of pullulan is shown in Figure 1.

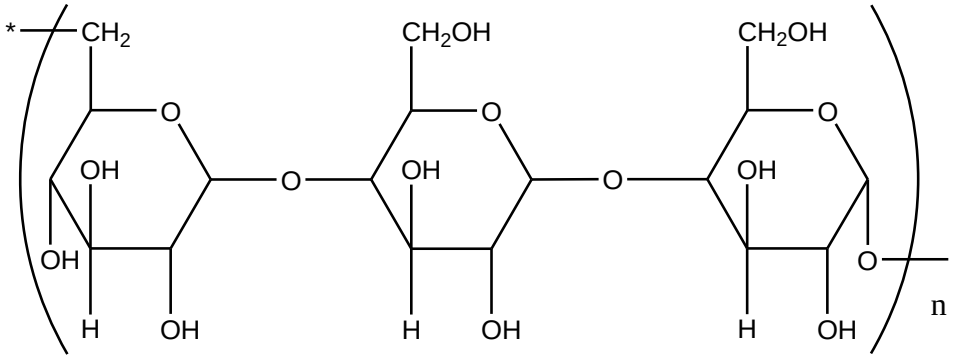


Figure 1. The maltotriose-repeating units of pullulan

Pullulan is white, odorless and tasteless powder. It can completely and readily dissolve in both cold and hot water. Except dimethylsulfoxide and dimethylformamide it is not soluble in organic solvents.

1.1.1 Physical Properties of Pullulan

Pullulan is a polysaccharide with different physical properties and it is mainly used for food-associated purposes. It is useful for producing water soluble products because of being water soluble. It has a very good film forming ability. Due to its film forming properties it is used as coatings on foods. Another physical property of pullulan is its ability to being compressed into tablets. (Lee, 2005)

The DSC thermogram of pullulan sample used in this study is shown in Figure 2. The glass transition temperature (Tg) is observed at 293°C.

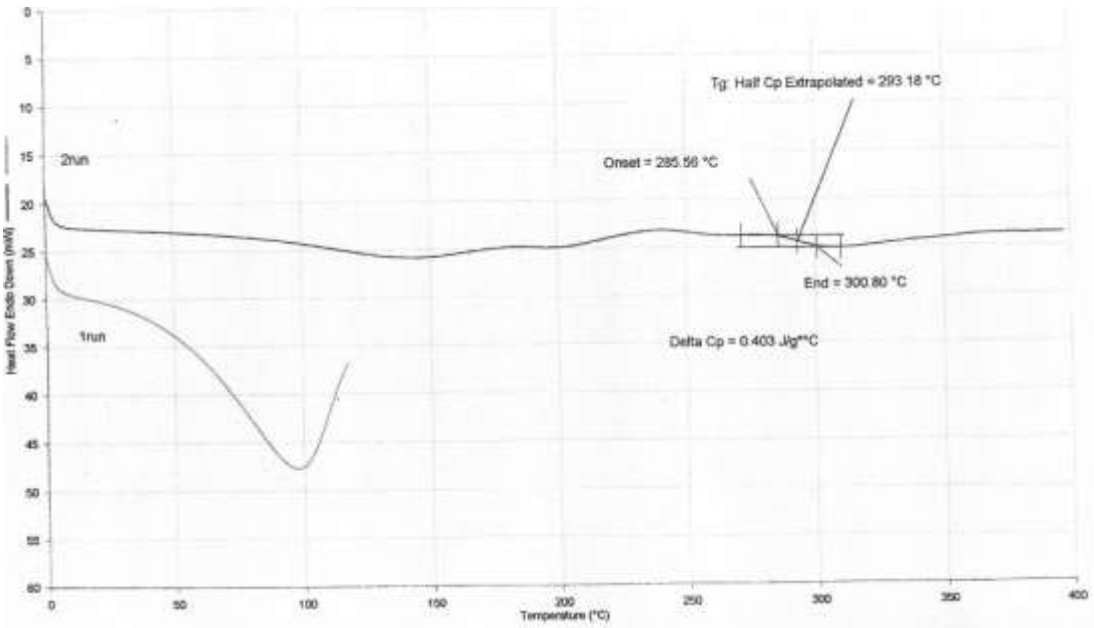


Figure 2. DSC thermogram of pullulan

1.1.2 Chemical Modification of Pullulan

Chemical modification of pullulan is possible via functional –OH groups available on the polymer backbone. Several different types of modification reactions were carried out on pullulan. For example, esterification and grafting was performed by using methyl acrylate (Wu, 2009), poly (methyl methacrylate) (Leonardis, 2010) and poly(ethylene glycol) (Jiao, 2003). Chlorination was achieved by using 3-acrylamidopropyl trimethylammonium chloride; it was shown that pullulan could be oxidized by using potassium persulfate as an initiator (Constantin 2011). Cholesteryl group was substituted to introduce hydrophobicity to this hydrophilic polymer. The derivatization efforts were mainly aimed at improving blood anticoagulant properties of pullulan. Physical, chemical and biological characteristics of pullulan used in this study as reported by the producer (Lee, 2005) are shown in Table 1.

Table 1. Physical, Chemical and Biological Characteristics of Pullulan produced by Hayashibara

Physical and Chemical Characteristics	
Generic name	Pullulan
Trade Name	HBC Pullulan
Chemical Family	Polysaccharide
Molecular Formula	$(C_6H_{10}O_5)_n$
Molecular weight range	100,000-200,000
Solubility	Water soluble
Biological Characteristics	
Degradability	Degradable
Toxicity	Safe

1.1.3 Pullulan Gels

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 epichlorohydrine. Crosslinked pullulan can be used to cure and heal infected wounds due to its action as an antibacterial agent and fluid adsorbent (Mocanu, 2002).

Hydrogel of pullulan was prepared by Autissier 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 desired shapes enable it to be used in vitro studies and vascular engineering (Autissier, 2006).

Pullulan can be easily modified by grafting different chemical structures on the backbone since it has available nine hydroxyl groups on the repeating unit (Figure 4). To combine advantages of both artificial and natural macromolecules, grafting molecules on regular polysaccharides such as chitosan and pullulan has been widely used.

1.1.4 Previous Grafting Studies on Pullulan

Studies on modification of pullulan via grafting of synthetic polymers are rather rare. Some examples that can be mentioned are grafting of 3-Acrylamidopropyl trimethylammonium chloride using potassium persulfate (KPS) as redox initiator (Constantin, 2011), grafting of carboxy terminated PEG onto pullulan via an esterification reaction (Jiao, 2003), grafting of poly (methyl methacrylate) onto amphiphilic pullulan copolymers (Leonardis, 2009) and grafting methyl acrylate onto pullulan (Wu, 2009)

1.1.4.1 Mechanism of Grafting 3-Acrylamidopropyl Trimethylammonium Chloride on Pullulan

Grafting of 3-Acrylamidopropyl trimethylammonium chloride on pullulan was carried out in aqueous media by using potassium persulphate (KPS) as the initiator which is considered as cheap and efficient agent (Constantin, 2011). The proposed grafting mechanism is shown below. According to (Ghimici, 2007) article, the flocculation efficiency of grafted pullulan by 3-acrylamidopropyl trimethylammonium chloride in clay suspension is studied.

$S_2O_8^{2-} \longrightarrow 2SO_4^{-\cdot}$

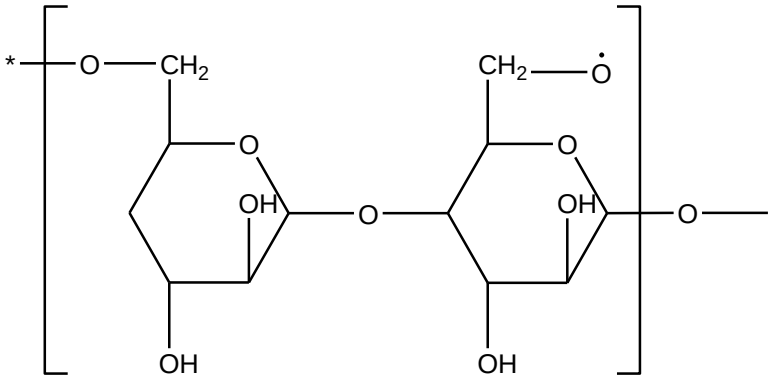
$SO_4^{-\cdot} + H_2O \longrightarrow HSO_4^- + HO^{\cdot}$

Initiation:

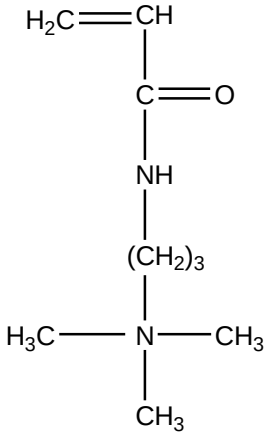
$M + R^{\cdot} \longrightarrow RM^{\cdot}$

$Pul + R^{\cdot} \longrightarrow PulO^{\cdot} + RH$

$R^{\cdot} = SO_4^{-\cdot}$ $HO^{\cdot}$



PluO·



M=APTAC

Propagation:

$PulO^{\cdot} + M \longrightarrow PulOM^{\cdot}$

$PulOM^{\cdot} + M \longrightarrow PulOM^{\cdot}_1$

$PulOM^{\cdot}_1 + M \longrightarrow PulOM^{\cdot}_2$

$PulOM^{\cdot}_{n-1} + M \longrightarrow PulOM^{\cdot}_n$

$RM^{\cdot} + M \longrightarrow RM^{\cdot}_1$

$RM^{\cdot}_1 + M \longrightarrow RM^{\cdot}_2$

$RM^{\cdot}_{n-1} + M \qquad RM^{\cdot}_n$

Termination:

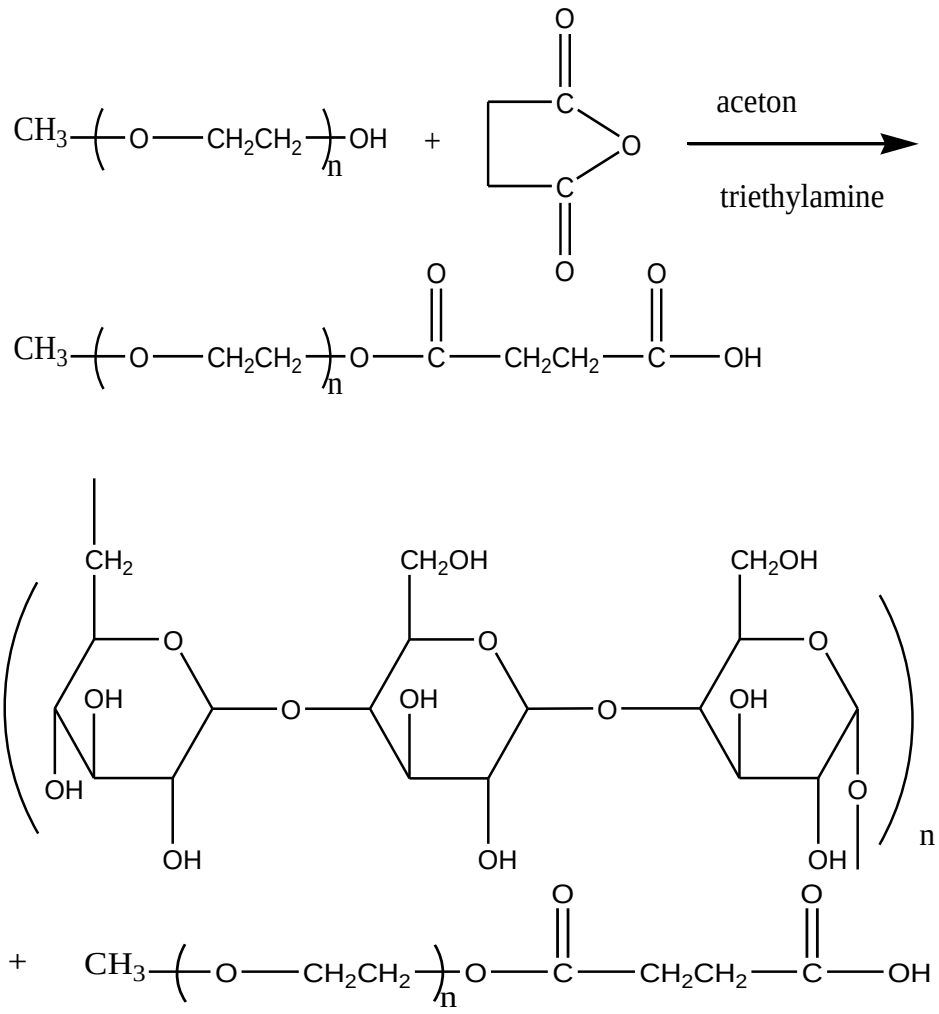
$PulOM^{\cdot}_{n} + PulOM^{\cdot}_{m} \longrightarrow$ Graft Copolymer

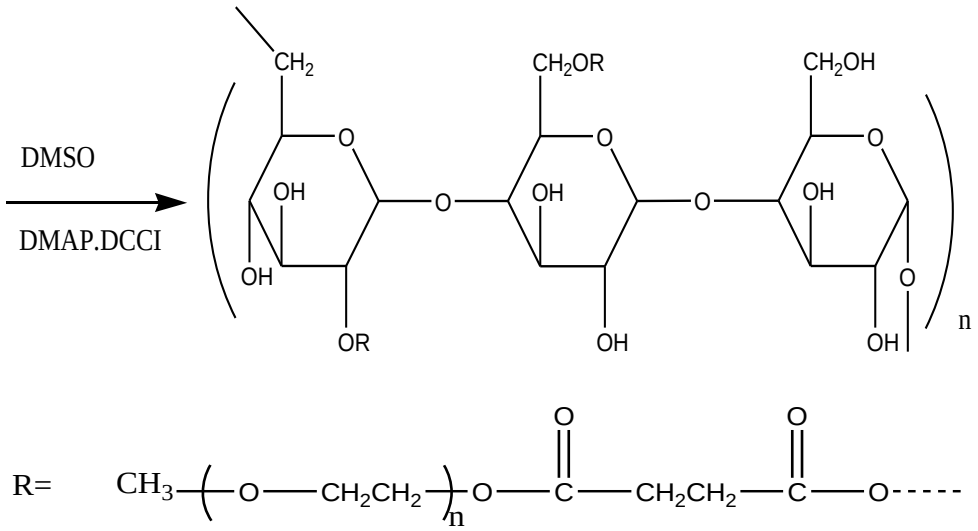
$PulO^{-}M^{\cdot}_{n} + RM^{\cdot}_{m} \longrightarrow$ Graft Copolymer

$RM^{\cdot}_{m} + RM^{\cdot}n \longrightarrow$ Homopolymer

1.1.4.2 Polyethylene glycol (PEG) Grafted on Pullulan

Carboxylic acid terminated PEG was grafted onto pullulan by Jiao *et al* in 2003 (Jiao 2003). The chemical reactions are shown below.





1.1.4.3 Grafted Poly (Methyl Methacrylate) onto Amphiphilic Copolymers of Pullulan

In a moderate homogeneous medium grafted of amphiphilic pullulan copolymers with PMMA were synthesized by Leonardis in 2009. It was discovered that pullulan can be easily grafted by atom transfer radical polymerization in lack of protecting group chemistry under homogeneous condition. Application of the mentioned amphiphilic copolymer is a drug delivery of hydrophilic molecules. (Leonardis, 2009)

1.2 N-Vinylimidazole

1.2.1 Chemistry of the N-Vinylimidazole

The chemical formula of N-Vinylimidazole is $C_5H_6N_2$ it has molar mass of 94.1145 g/mol. This molecule also named as vinyl imidazole, 1H imidazole, 1-ethenyl, 1-vinyl imidazole. The chemical structure of N-vinyl imidazole (NVI) is shown in Figure (3).

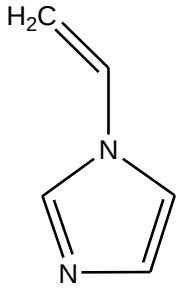


Figure 3. The chemical structure of N-Vinylimidazole

1.2.2 N-Vinylimidazole Grafted Polysaccharides and their Applications

Grafting N-vinylimidazole on carboxymethyl chitosan was carried out by Sabaa under the following reaction condition:Temperature= 60°C, Time= 2.5h in aqueous solution by using potassium persulphate (KPS) as initiator to produce a high thermal stability polymer (Sabaa, 2010). The thermogravimetric analysis (TGA) shows thermal stability of the copolymer (CMCh-g-PNVI) is better than CMCh, it means by increasing grafting percentage polymer thermal stability increase.

The product was completely soluble in distilled water and acetic acid but it was mainly soluble in (1:1) acetic acid: ethanol solution. It was insoluble in ethanol, 1,4-dioxane, DMF and THF. Caner *et al* (2007) have grafted poly N-vinylimidazole onto chitosan. The polymerization reaction carried out under nitrogen atmosphere at 70°C per 3h in dilute acetic acid solution with ceric ion initiation. Two different chitosan samples were used. One of them has 85% degree of deacetylation and the other one has 90% degree of deacetylation. The product found soluble in distilled water and acetic acid but insoluble in DMF, DMSO, THF and ethanol. In glacial acetic acid and ethanol the polymer swelled.

1.3 Aim of the Thesis

The aim of this thesis is to establish the suitable conditions for grafting PNVI onto pullulan by redox initiation. Pullulan itself is a neutral polysaccharide freely soluble in water. PNVI on the other hand is a synthetic polymer with water solubility similar to pullulan.

One characteristic of PNVI different than those of pullulan is its cationic nature in aqueous acid medium due to the chemical structure of the imidazole group. It is anticipated to obtain the following graft copolymer which will become protonated in aqueous acid medium.

The products would have a potential to be applied as pH-responsive drug delivery system. They would also bear complexation capacity with polyanions such as DNA and would have a potential to be applied as non-viral gene delivery systems.

Chapter 2

EXPERIMENTAL

2.1 Materials

The chemicals used in this study are shown in Table 2.

Table 2. Chemicals Used in the Study

NO	Chemical	Manufacturer
1	Pullulan	Hayashibara-Japan
2	1-Vinyl imidazole	Aldrich-Germany
3	Potassium persulphate	Aldrich-Germany
4	Ethanol	SAFA-North Cyprus
5	Acetone	TEKIM-North Cyprus
6	Toluene	Sigma-Germany
7	Ceric ammonium nitrate	Aldrich-Germany
8	Hydrochloric acid	Sigma-Germany
9	Sodium hydroxide	Aldrich-Germany

2.2 Methods

2.2.1 Grafting under Homogenous Conditions

Pullulan was weighed by using an analytical balance. The weighed pullulan was dissolved in 20 mL of distilled water under magnetic stirring for 30 minutes to obtain a homogenous solution. N-vinylimidazole (NVI) and potassium persulphate (KPS) 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. Then, the solution was poured into acetone with vigorous stirring to precipitate the product. The precipitate was filtered, washed with ethanol for removal of the homopolymer, and then was dialyzed against distilled water to remove any unreacted potassium persulfate, any unreacted initiator or other impurities. A regenerated cellulose Spectra/Por (RC) dialysis membrane with 6000-8000 MWCO was used. The dialysis was carried out overnight. It was then dried at 50 °C overnight. The color of all samples was white. Grafting conditions applied in the homogenous system are shown in Table 3.

Table 3. Grafting conditions in homogenous system

ID	Pullulan (g)	KPS (g)	NVI (mL)	Duration (h)	Temperature (°C)
S1	1.0012	0.1330	0.270	2	35
S2	1.0018	0.2661	0.270	2	35
S3	1.0028	0.2715	0.270	3	35
S4	1.0021	0.2638	0.270	4	35
S5	1.0065	0.2661	0.270	2	35
S6	1.0143	0.2661	0.270	2	40
S7	1.0016	0.2661	0.270	2	50
S8	1.0068	0.2660	0.135	2	40
S9	1.0300	0.2660	0.540	2	40
S10	0.2031	0.2690	0.270	2	40
S11	0.2057	0.1343	0.270	2	40
S12	1.0081	0.1330	0.270	2	40
S13	1.0071	0.2670	0.270	2	70
S14	1.0079	0.2710	0.270	3	70

2.2.2 Grafting under Heterogeneous Conditions

Pullulan powder was weighed into 100 mL of toluene in three neck round bottom flask. A fixed temperature water bath was ready; the flask was put into the water bath. To remove oxygen gas from the system, nitrogen gas was passed into the system for around 30 minutes. A given amount of cerium ammonium nitrate (CAN) was dissolved in 5 mL ethanol to be added to the system as an initiator. After 15 minutes a given amount of monomer, N-vinylimidazole (NVI) was added to the system. To avoid evaporation of toluene, the reaction was carried out under reflux. When the predetermined hour of reaction passed, the grafted product was taken out from the flask by filtration; the collected product was washed in ethanol to remove any stuck homopolymers into the sample, and then the sample was dialyzed against distilled water for removal potassium persulfate, any unreacted initiator or other impurities. The dialysis was carried out overnight. The sample was dried at 40 °C.

The color of all samples was yellow. The color became darker approaching brown with increasing grafting yield. Different reaction conditions applied in heterogeneous system is shown in Table 4.

Table 4. Grafting conditions in heterogeneous system

ID	Pullulan (g)	NVI (mL)	CAN (g)	Time (h)	Temp, (°C)
S_1	0.1000	5	0.5	1	60
S_2	0.1000	10	0.5	1	60
S_3	0.5000	10	0.5	1	60
S_4	0.1226	15	0.5	1	60
S_5	0.1114	10	1	1	60
S_6	0.1280	10	0.5	1	45
S_7	0.1120	10	0.5	1	70
S_8	0.1169	10	0.5	2	60

Table 5 shows other samples prepared for comparison such as blank samples under homogenous and heterogeneous conditions, and one grafted sample under homogenous conditions using CAN as the initiator and one grafted sample under heterogeneous conditions by using KPS as the initiator.

Table 5. Blank samples and samples used different initiator

ID	Condition	Pullulan (g)	NVI (mL)	CAN (g)	KPS (g)	Color
S_1	Homogenous	0.2191	-	-	0.2822	White
S_2	Homogenous	0.2034	-	0.2784	-	Yellow
S_3	Heterogeneous	0.491	-	2.5161	-	Transparent
S_4	Heterogeneous	0.2138	-	-	0.271	White
S_5	Homogenous	0.2508	0.27	0.2909	-	Yellow
S_6	Heterogeneous	0.1114	10	-	0.5487	White and Yellow

2.3 Solubility

2.3.1 Water Solubility

Since both the monomer (NVI) and pullulan are completely soluble in water, solubility of products in water was assumed. To check the solubility of products in water, 20 mL of distilled water at room temperature was poured in a beaker and then 0.05 g of product was added under magnetic stirring. After 24 h the water solubility of products were observed.

2.3.2 Acid Solubility

To study the solubility of samples in acid, 0.05 g of each sample was placed into 20 mL of 0.1 M hydrocholoric acid at room temperature. Then after 24 hours the solubility of each sample was observed.

2.4 Characterization

The products were characterized by FTIR, UV, C-13 NMR spectroscopies and by DSC and elemental analyses.

2.4.1 FTIR Analysis

The device used to record FTIR spectra of samples is Perkin Elmer spectrum-65.

2.4.2 Ultraviolet-Visible Spectroscopy

The UV spectra of sampels were recorded by T80+UV/VIS spectrometer.

2.4.3 DSC Analysis or Different Scaning Calorimetry

The DSC analysis of samples was carried out at TUBITAK-MAM Gebze Turkey.

2.4.4 Carbon-13 NMR Analysis

The products were analysed by Carbon-13 NMR at METU (ODTÜ MerkezLaboratuvarı) in Ankara, Turkey.

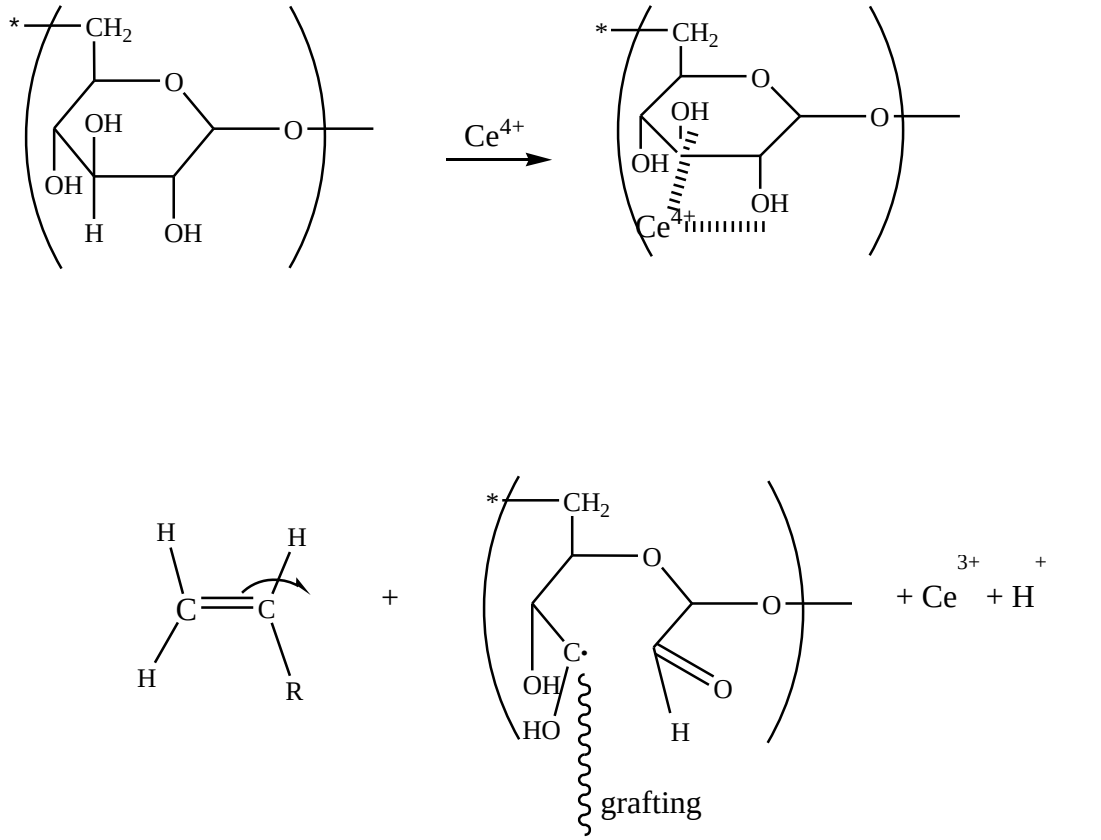
Chapter 3

RESULTS AND DISCUSSION

The conditions for grafting poly (N-Vinylimidazole) onto pullulan were investigated. Two different redox initiators, namely cerium (IV) ammonium nitrate (CAN) and potassium persulphate (KPS) were tested for this purpose under homogeneous and heterogeneous conditions. The samples were characterized using spectroscopic methods, solubility tests, and gel permeation chromatography.

3.1 Reaction Conditions Investigated for Grafting PNVI onto Pullulan

In this section the gravimetric results obtained under homogenous and heterogeneous conditions are presented. Pullulan was grafted with PNVI using CAN or KPS as redox initiator. Two of the possible structures are shown below:



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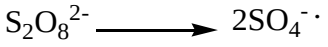
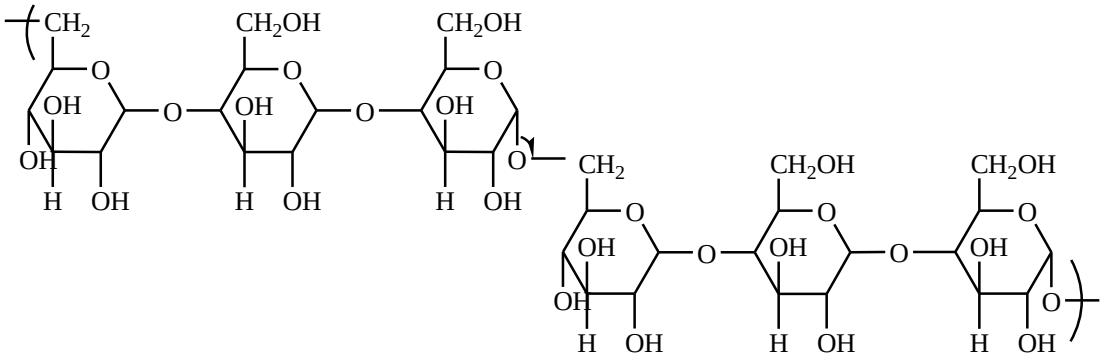


SO4- .



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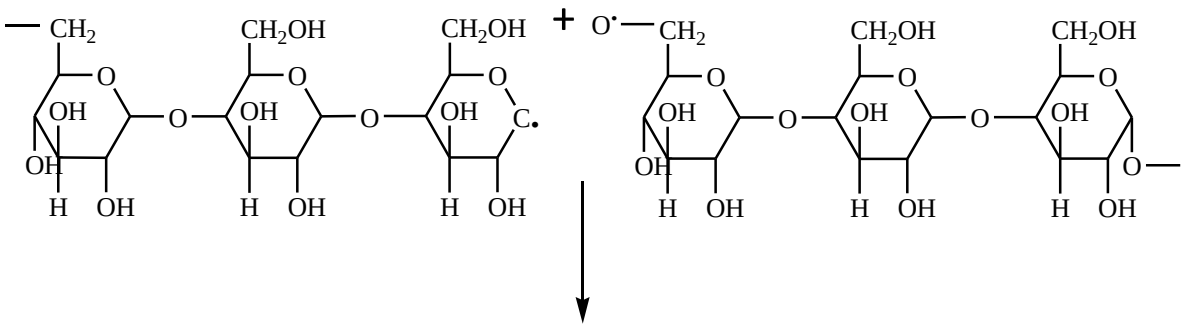

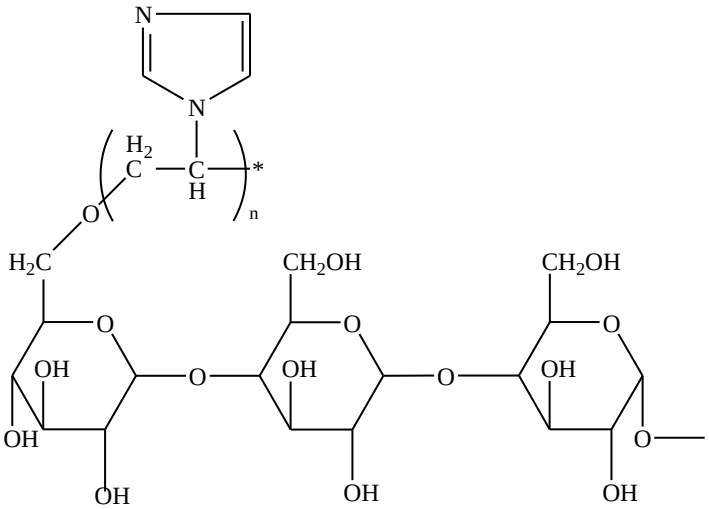

3.1.1 Homogeneous Conditions

For grafting of PNVI onto pullulan under homogeneous conditions water was chosen as the solvent. All reactants namely the monomer (NVI), the substrate (pullulan), the initiator CAN or KPS are all soluble in water. The grafting conditions and grafting yields (%G) are summarized in Table 6.

Table 6. Grafting yields under homogenous conditions

ID	Pullulan (g)	KPS (g)	NVI (mL)	Duration (h)	CAN (g)	Temperature (°C)	$\%G_1$ *	$\%G_2$ **	Color
S1	1.0012	0.133	0.27	2	-	35	33.5	N/A	White
S2	1.0018	0.2661	0.27	2	-	35	36.1	N/A	White
S3	1.0028	0.2715	0.27	3	-	35	30.2	-	White
S4	1.0021	0.2638	0.27	4	-	35	-	N/A	White
S5	1.0065	0.2661	0.27	2	-	35	30.4	N/A	White
S6	1.0143	0.2661	0.27	2	-	40	42.7	-	White
S7	1.0016	0.2661	0.27	2	-	50	17.8	N/A	White
S8	1.0068	0.266	0.135	2	-	40	11.6	N/A	White
S9	1.030	0.266	0.54	2	-	40	14.8	9.7	White
S10	0.2031	0.269	0.27	2	-	40	84.9	4.5	White
S11	0.2057	0.1343	0.27	2	-	40	29.3	N/A	White
S12	1.0081	0.133	0.27	2	-	40	2.26	N/A	White
S13	1.0071	0.267	0.27	2	-	70	N/A	N/A	White
S14	1.0079	0.271	0.27	3	-	70	N/A	N/A	White
S15	0.2191	0.2822	-	2	-	35	101	-	White
S16	0.2034		-	2	0.2748	35	114	-	Yellow
S17	0.2508	-	0.27	2	0.2909	35	67.5	36.6	Yellow

*$\%G_1$ is the grafting percentage before dialysis against distilled water
** $\%G_2$ is the grafting percentage after dialysis against distilled water

As can be followed from Table 6, several different amounts of pullulan (1.0 g, 0.2g), KPS (0.1 g, 0.2 g) and NVI (0.1 mL, 0.2 mL and 0.5 mL) were used for different reaction times (2 h, 3 h and 4 h) and at different reaction temperatures (35 °C, 40 °C, 50 °C and 70 °C) to test for the possibility of grafting PNVI onto pullulan.

A blank pullulan sample (S15) treated with KPS under similar conditions as grafted samples gave a % increase in weight of 101.6% even in the absence of the monomer NVI. Furthermore a blank sample of pullulan treated with CAN (S16) also gave a similar result with an increase in weight by 114.3%. These results imply that the products obtained after reaction, which were washed with ethanol to remove any homopolymer formed, were not free of any other impurities. It was observed that the color of the samples treated with KPS was white while the samples treated with CAN (S16 and S17) were yellow in color. The yellow color of the samples obtained when CAN is used as the redox initiator is one evidence for the presence of insoluble cerium salt impurities in the samples and/or formation of cerium complexes with the product. A similar finding was previously reported by Caner et al (Caner, 1998) in an article reporting grafting of poly(4-vinylpyridine) onto chitosan.

Another possibility leading to weight gain even in the absence of the monomer could be the oxidation of pullulan. These possibilities were tested by further characterization as explained as follows.

The samples were dialyzed against distilled water to find out whether there was any unreacted initiator and/or monomer or oligomers of PNVI left after washing the products with ethanol. The results obtained are shown in Table 6 as $\%G_2$. It can be observed that no significant weight gain could be detected after dialysis except for the sample S17. Hence, any unreacted KPS or NVI that are freely soluble in water were cleaned from the products. However, it should also be noted that pullulan is prone to mechanical degradation in solution. Therefore it is highly probable that some grafted product or ungrafted pullulan might also have been degraded and escaped into dialysis water.

Therefore it was concluded that the weight gain obtained before dialysis could have been due to the presence of unreacted monomer or initiator, in the case of using KPS as the initiator. The attempt to purify the product from unreacted initiator or monomer by dialysis against water resulted in loss of the product due to the vulnerability of pullulan to solution degradation by chain scission. If the initiator was CAN, in addition to degradation there was also a possibility of having insoluble impurities in the products. Hence, homogenous conditions are not suitable for grafting PNVI onto pullulan. One reason is the slow polymerization of NVI in aqueous medium when pH is more than 6 (Santanakrishnan, 2013). Due to degradative addition to monomer at higher pH values as shown below.

3.1.2 Heterogeneous Conditions

Since homogenous conditions did not provide any solid product, heterogeneous conditions were investigated as a second alternative. For grafting PNVI onto pullulan under heterogeneous conditions toluene was chosen as the solvent and to avoid evaporation of toluene during grafting, the reaction was carried out under reflux. The advantage of toluene is that both the monomer and the polymer, PNVI are soluble in this solvent. Any homopolymer formed remains in solution. Consequently, the product is relatively clean from the homopolymer except for some PNVI adsorbed on the product. To remove any unreacted monomer and initiator and any oligomer of PNVI from the products, dialysis against distilled water was carried out as in the first case explained above. The grafting conditions and grafting yields (%G) are summarized in Table 7. The grafting percentage before dialysis is given as ($\%G_1$) and the grafting percentage after dialysis is ($\%G_2$).

Table 7. Grafting yields under heterogeneous conditions

ID	Pullulan (g)	NVI (mL)	KPS(g)	CAN(g)	Time (h)	Temp, (°C)	$\%G_1$ *	$\%G_2$ **	Color
S_1	0.1000	5	-	0.5	1	60	9.6	N/A	Yellow
S_2	0.1000	10	-	0.5	1	60	165	103	Yellow
S_3	0.5000	10	-	0.5	1	60	19.5	-	Yellow
S_4	0.1226	15	-	0.5	1	60	40.4	N/A	Yellow
S_5	0.1114	10	-	1.0	1	60	107	N/A	Yellow
S_6	0.1280	10	-	0.5	1	45	44.4	N/A	Yellow
S_7	0.1120	10	-	0.5	1	70	119.6	N/A	Brown
S_8	0.4910	-	-	2.5161	1	60	N/A	N/A	Transparent
S_9	0.2138	-	0.271	-	1	60	N/A	N/A	Transparent
S_{10}	0.1114	10	0.5487	-	1	60	1221	162	White and Yellow

*$\%G_1$ is the grafting percentage before dialysis
** $\%G_2$ is the grafting percentage after dialysis

As it shown in Table 7 to optimize the grafting yield (%G) different amount of pullulan (0.1 g, 0.5 g), CAN (0.1 g, 0.5 g) and NVI (5 mL, 10 mL and 15 mL) were used under different reaction times (1 h, 2 h) and different reaction temperatures (45°C, 60°C and 70°C). The grafting yields before dialysis ($\%G_1$) were changed between 9.6% and 165.5%. The grafting yields after dialysis were changed between 0% and 103.2%. The highest %G was obtained as 103% using 0.1 g pullulan, 10 mL NVI, 0.5 g CAN under 60°C during 1 hour (S_2) and as 162% using 0.1114 g pullulan, 10 mL NVI, 0.5484 g KPS under 60°C during 1 hour (S_{10}).

Under heterogonous conditions a suitable medium was available for the polymerization of NVI. The monomer can easily polymerize in organic solvents such as toluene or benzene (Santanakrishnan,2013). Also degradation of pullulan has been prevented to a certain degree.

3.2 Characterization

The products were characterized by ultraviolet-visible spectroscopy, FTIR spectroscopy, Carbon-13 NMR spectroscopy, and GPC analysis.

3.2.1 Ultraviolet-Visible Spectroscopy

The UV spectrum of the product obtained under homogeneous conditions using KPS as the initiator (sample S9), sample (S15) which is KPS treated pullulan, Pullulan, product obtained using CAN as the initiator under homogeneous conditions (sample S17), sample (S16) which is CAN treated pullulan under homogeneous conditions, sample (S_{10}), the product obtained using KPS as the initiator under heterogeneous conditions, NVI, and product obtained using CAN as the initiator under heterogeneous conditions namely sample (S_2) after dialysis are shown in Figure 5 as (a), (b), (c), (d), (e), (f), (g), and (h) respectively. The absorption of NVI, PNVI and pullulan are shown in Table 8. The UV spectra were taken in 0.1 M HCl solution.

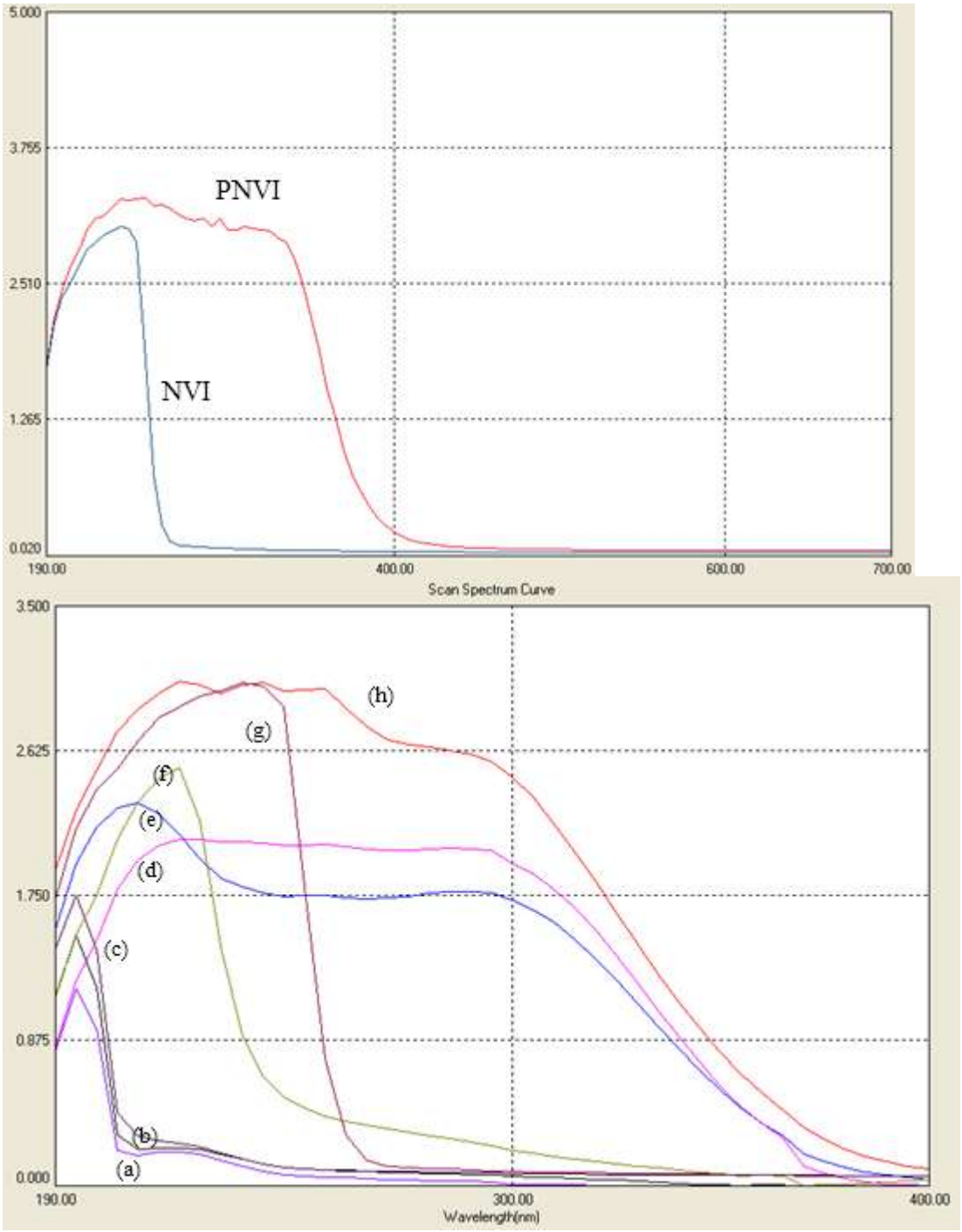

Figure 4. UV Spectra of (a) S9, (b) S15, (c) Pullulan, (d) S17, (e) S16, (f) S_{10}, (g) NVI, (h) S_2 after dialysis

Table 8. UV absorptions of NVI, PNVI and Pullulan

Sample	**Absorptions**	**Transition of**	
NVI	210 nm 230 nm	Π-δ* Π- Π*	C=N of the Imidazole C=N
PNVI treated by CAN	210 nm, 220 nm 230 nm 320 nm broad	Π-δ* Π- Π*	C=N of the Imidazole C-N Cerium complexes
Pullulan	220 nm	n- Π *	-CH2 alkane Primary alcohol Secondary alcohol

Figure 5 (a), (b) and (c) which belong to the 'grafted product' obtained under homogeneous conditions using KPS as the initiator (sample S9), sample (S15) which is KPS treated pullulan, and pullulan respectively are identical. They all absorb at 200 nm together with a weaker absorption at 230 nm. This observation tells us that pullulan does not undergo any chemical change after being treated with KPS under given experimental conditions whether in the absence or presence of NVI. Hence, grafting of PNVI onto pullulan could not have been achieved under homogeneous conditions using KPS as the initiator.

When Figure 5 (d), (e) and (h) are compared to each other and to the spectrum of NVI shown in Figure 5 (g), the following observations can be made: CAN-treated pullulan (e) and the 'grafted product' obtained homogeneous conditions using CAN as the initiator are similar with broad absorptions at 220-230 nm region and at 290

nm. Hence, it can be concluded that graft copolymerization of PNVI onto pullulan was not achieved using CAN instead of KPS as initiator under homogeneous conditions.

The changes in the UV spectrum of CAN treated pullulan (e)compared to that of pullulan (c) or KPS treated pullulan (b) suggest that pullulan should form complexes with Ce^{4+}/Ce^{3+} ions present in the medium.

NVI, on the other hand, absorbs at 210 nm and 230 nm. PNVI prepared by CAN initiation absorbs at 210 nm, 220 nm, 230 nm and 320 nm (broad). 210 nm and 230 nm absorptions of PNVI comes from NVI. The other absorption should be due to the complex formed with cerium.

When the uv spectrum of the 'grafted sample' obtained under heterogeneous conditions using CAN as the initiator (h) is examined absorptions at 220 nm, 230 nm, 240 nm and 290 nm can be identified. The spectrum contains features of CAN treated pullulan and NVI or PNVI prepared by CAN initiation, indicating grafting of PNVI onto pullulan successfully. The grafted sample contains absorptions due to the presence of Ce salts or complexes even after dialysis. These complexes with Ce^{4+}/Ce^{3+} ion are not ethanol soluble or water soluble. That is why washing with ethanol or dialysis against distilled water did not remove the complexes from the products. It should be noted that the color of CAN-treated pullulan or grafted pullulan is yellow as a visible indication of complex formation, or presence of cerium salts. The 'grafted product' obtained under heterogeneous conditions using KPS as initiator represented by Figure 5 (f), shows absorption at 200 nm as a shoulder followed by a strong absorption at 230 nm indicating a structure

compromising pullulan and NVI characteristics. Hence, successful grafting of PNVI onto pullulan under heterogeneous conditions using KPS as the initiator and toluene as the solvent at 60 C for one hour reaction time has been achieved. These findings have been confirmed by FTIR analysis as will be explained in the following section.

From the UV analysis the following conclusions can be drawn:

1. pullulan forms complex with Ce^{4+}/Ce^{3+} ion.
2. PNVI also forms complex with Ce^{4+}/Ce^{3+} ion.
3. No grafting occurs under homogeneous conditions whether the initiator is KPS or CAN.
4. Grafting occurs under heterogeneous condition whether the initiator in KPS or CAN.
5. The products with using CAN as an initiator, contain Ce^{4+}/Ce^{3+} incorporated into the system via complexation or by salt formation.
6. The product obtained under heterogeneous conditions using KPS as an initiator, a clean pullulan-*graft*-PNVI product is obtained.

3.2.2 FTIR Analysis

In Figure 6 the FTIR spectrum of pullulan, sample (S17), PNVI, sample (S_2), sample (S_{10}) are shown respectively. In the FTIR spectrum of pullulan (6a), 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 bounds of the polymer are observed at 1148 cm^{-1}, 1078 cm^{-1}, 995 cm^{-1} and 929 cm^{-1}. In Figure 6 (b), in the FTIR spectrum of the sample (S17) prepared under homogenous conditions using CAN as the initiator there is no evidence for the grafting of PNVI. The spectrum of pullulan shown in Figure 6 (a) and the spectrum of (S17) shown in Figure 6 (b) are almost identical.

The FTIR spectrum of PNVI homopolymer is given in Figure 6 (c). The peaks observed 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}.

The spectra of the grafted products prepared under heterogeneous conditions using CAN (S_2) and KPS (S_{10}) as an initiator are shown in Figure 6 (d) and (e) respectively. The characteristics of both pullulan and PNVI can be observed in both spectra. In addition to glycosidic and etheric bound of pullulan in the region 1100-900 cm^{-1}, characteristic C=N stretching bounds of PNVI can be observed at 1540-1570 cm^{-1} region in both spectra. Therefore, formation of pullulan-*graft*-PNVI under heterogeneous conditions is further confirmed by FTIR analysis in addition to UV spectroscopy.

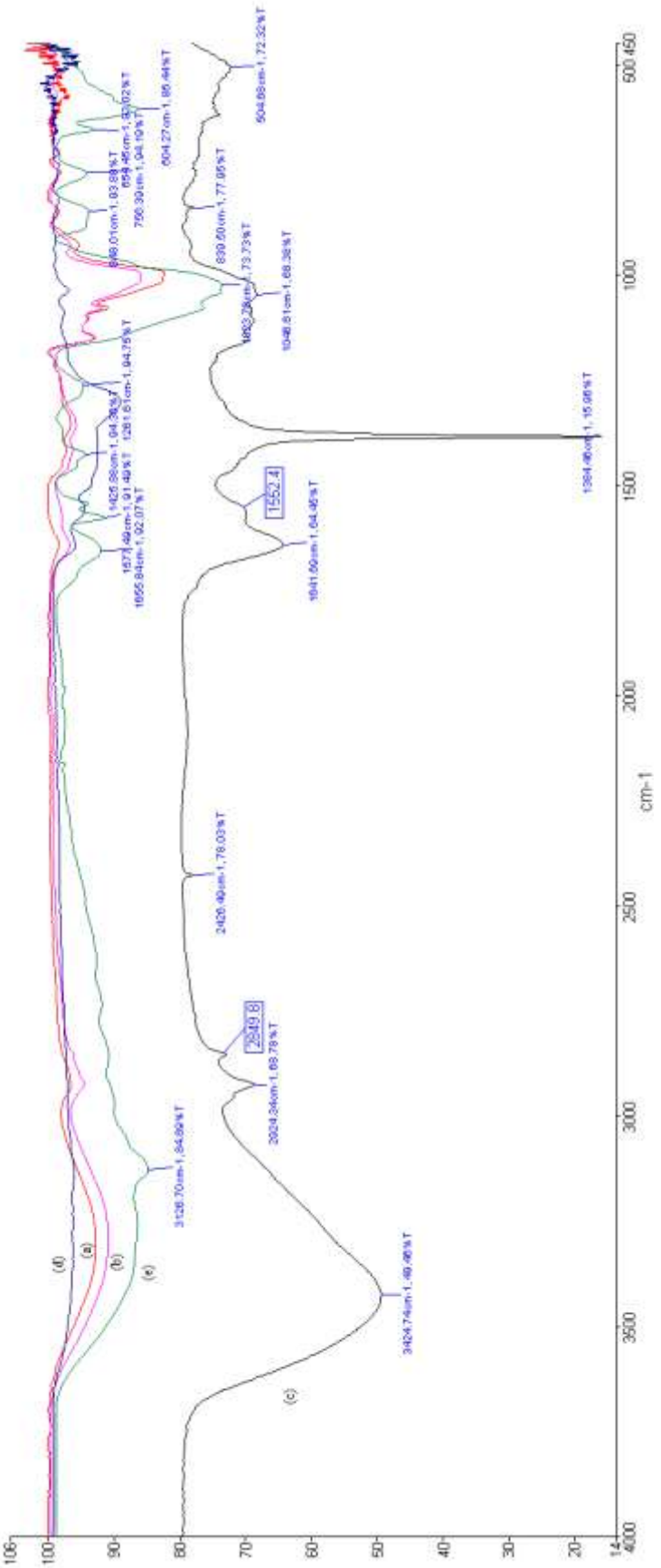

Figure 5. FTIR spectrum of (a) pullulan,(b) sample (S17), (c) PNVI, (d) sample (S2), (e) sample (S10)

3.2.3 Carbon-13 NMR Analysis

CP-MAS C-13 NMR spectrum of pullulan and sample (S10) is shown in Figure 9. These spectra give further evidence to the fact that this is no grafting under homogenous conditions. Sample (S10) was prepared in solution using KPS as initiator. As it can be followed from Figure 7, (a) and (b), both C-13 spectra are identical. Hence graft of PNVI onto pullulan was not succesful under the given conditions. Also, there is no evidence for oxidative degradation. Pullulan treated with KPS has no chemical change in structure. When these results are considered together with GPC results given below, it can be concluded that pullulan undergoes chain scission in aqueous solution by the action of the redox initiator.

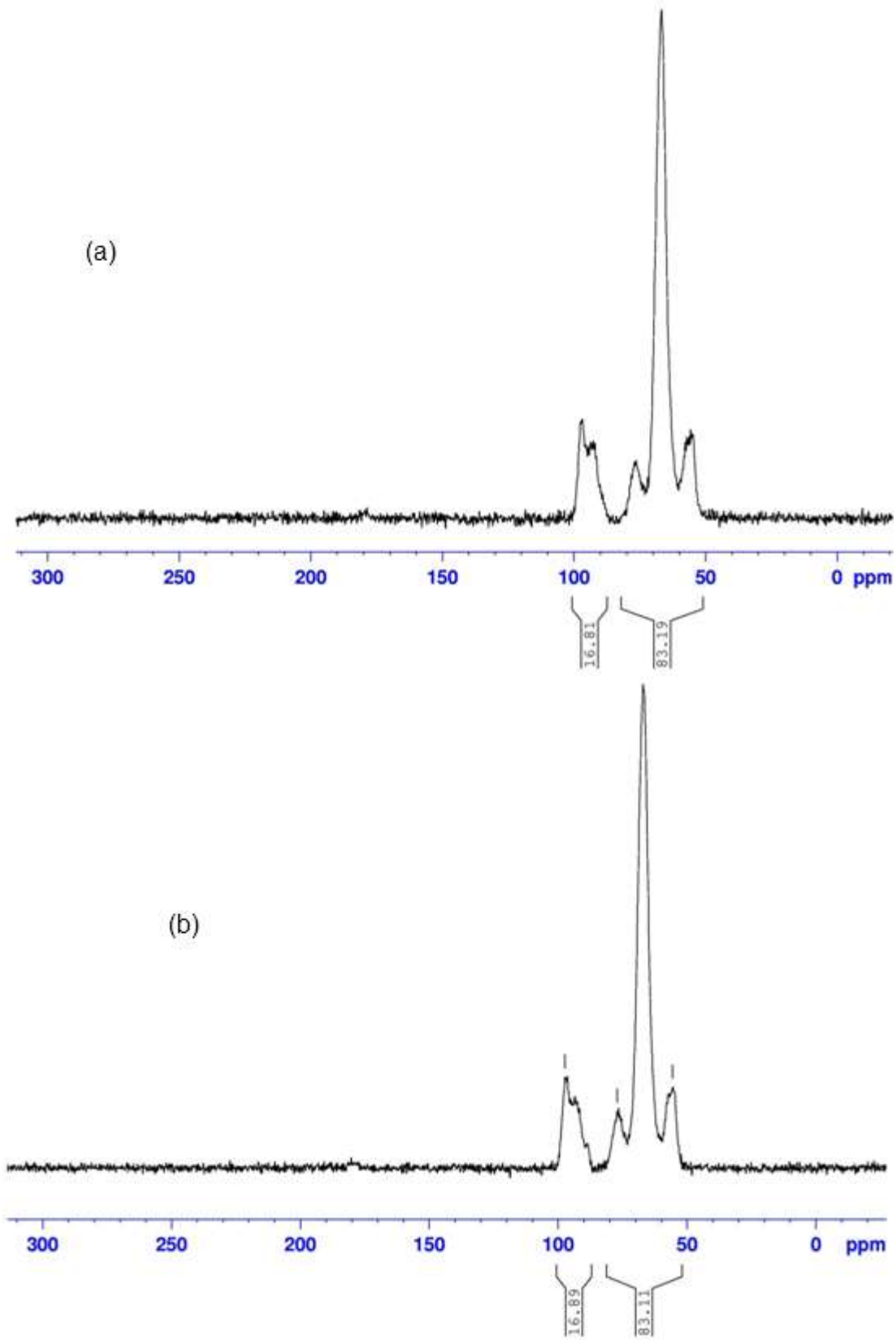

Figure 6. Carbon-13 NMR analysis of (a) pullulan and (b) sample (S10)

3.2.4 GPC Analysis

Figure 8 shows GPC curves for pullulan, S6 and S10. 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 GPC. Two other samples (S6 and S10) treated with potassium persulphate at 40°C for 2 hours 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. No grafting was achieved but pullulan degraded. As KPS/pullulan ratio increases molecular weight decreases as shown in Table9.

Table 9. GPC analysis results for pullulan, S6 and S10

Sample	**no of mole of KPS/repeat unit**	**M_n**	**M_w**	**M_p**	**PDI**
Pullulan	-	154700	347000	214800	2.24
S6	2.7	92800	180700	132100	1.95
S10	2.0	26800	52400	44300	1.95

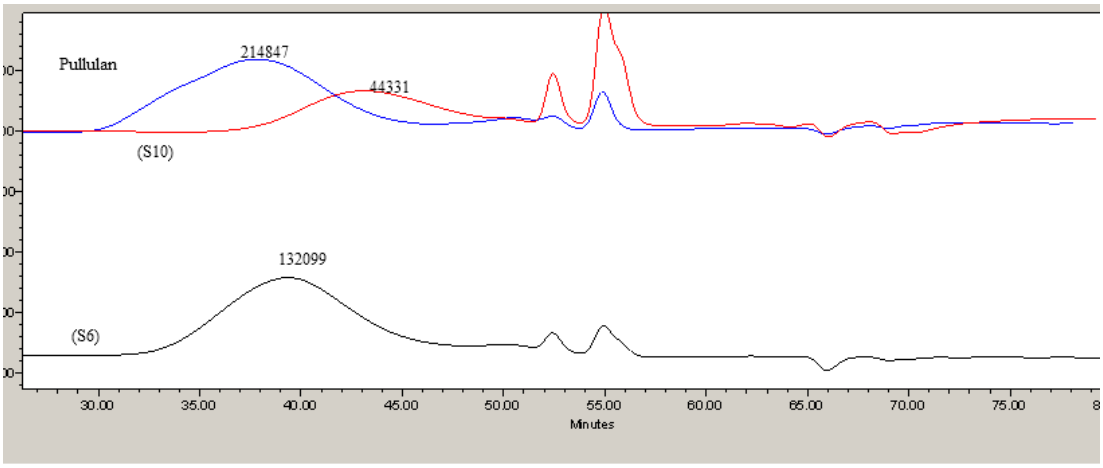

Figure 7. GPC curves for pullulan, S6 and S10

3.2.5 Solubility Tests

The products were tested for their solubility in distilled water and in 0.1 M HCl solution. The results are shown in Table 10. While CAN initiated grafted product was only partially soluble in acid solution, KPS initiated one exhibited better dissolution in the same solvent. This difference should be due to the presence of insoluble complexes in the CAN initiated product. Similarly PNVI obtained by CAN initiation is only partially soluble.

Table 10. Solubility test results

Sample	**Water**	**0.1 M HCl**
Pullulan	Soluble	Soluble
Degraded Pullulan-CAN	Partially Soluble	Partially Soluble
Degraded Pullulan-KPS	Soluble	Soluble
Grafted product-CAN	Partially Soluble	Partially Soluble
Grafted product-KPS	Soluble	Soluble
PNVI-CAN initiated	Partially Soluble	Partially Soluble

Chapter 4

CONCLUSION

PNVI grafting onto pullulan was achieved using potassium persulphate or cerium ammonium nitrate initiation under heterogeneous conditions. KPS is a more useful initiator than CAN as the grafted product obtained using KPS did not contain any impurities after dialysis against water. The disadvantage of CAN as redox initiator in *graft* copolymerization onto pullulan is that it forms insoluble salts or complexes during grafting reaction which cannot be separated from product.

Pullulan is not a durable substrate for graft copolymerization in solution in homogeneous conditions as it undergoes chain scission in aqueous solution.

Grafting reaction was successful under heterogeneous conditions, which were carried out, in an organic solvent, toluene. Toluene is a better solvent than water for NVI polymerization. This factor should have contributed to the more successful grafting reaction carried out under heterogeneous conditions. In water, degradative chain transfer to monomer hinders polymerization of NVI and hence grafting is hindered.

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