DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

PREPARATION AND CHARACTERIZATION OF CROSSLINKED CHITOSAN BASED HYDROGELS

by
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İZMİR

PREPARATION AND CHARACTERIZATION OF CROSSLINKED CHITOSAN BASED HYDROGELS

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We have read the thesis entitled **"PREPARATION AND CHARACTERIZATION OF CROSSLINKED CHITOSAN BASED HYDROGELS"** completed by **BEHİYE ÖZTÜRK** under supervision of **PROF. DR. KADİR YURDAKOÇ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Behiye ÖZTÜRK

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ABSTRACT

An interpenetration network (IPN) ion-imprinting membrane was synthesized using silver ions as template for adsorption and removal of silver ions from aqueous solutions. The membrane was obtained via cross linking of chitosan (CS), polyvinyl alcohol (PVA), and blend chitosan/polyvinyl alcohol using glutaraldehyde (GA) as cross linker. Non-ion imprinted membrane was also synthesized. Chitosan and polyvinyl alcohol are not very compatible in the chitosan/ polyvinyl alcohol blended hydrogel membrane and the thermostability of the membranes is enhanced by glutaraldehyde as crosslink agent. Later synthesis, the membranes were immersed in 1 M sodium hydroxide, followed by repeatability washing with deionized water to eliminate any unreacted glutaraldehyde. Quality analysis was applied on the samples by using Tollen reagent for the determination of the unreacted glutaraldehyde. The characterization with different methods (FTIR, TGA, XRD, and SEM) for non-imprinted and silver ion imprinted membrane were examined. FT-IR analysis implies that the coordinating atoms may be nitrogen atom of amino in chitosan and oxygen atom of hydroxide in polyvinyl alcohol. Morphology and properties of silver ion-imprinted membranes were studied compared with non-imprinted and chitosan. The silver-imprinted membranes can be regenerated by using ethylenediaminetetraacetic acid and thiourea in hydrochloric acid. The amounts of silver-ions were determined by the atomic absorption spectroscopic analysis. As a result, it was found that thiourea in hydrochloric acid solution had higher removal capacity for silver (I) ions when compared with ethylenediaminetetraacetic acid.

Keywords: chitosan, ion-imprinting membrane, chitosan/PVA, silver removal

ÇAPRAZ BAĞLANMIŞ CHİTOSAN TABANLI HİDROJELLERİN KARAKTERİZASYONU VE HAZIRLANMASI

ÖZ

Sulu çözeltilerden gümüş iyonlarının uzaklaştırılması ve adsorpsiyon için şablon olarak gümüş iyonlarının kullanılacağı iyon baskılayıcı membran sentezlenmiştir. Bağlayıcı olarak glutaraldehitin kullanıldığı kitosan (CS), polivinil alkol (PVA) ve kitosan/polivinil alkol karışımının çapraz bağlanmasıyla membran elde edilmiştir. Ayrıca iyon baskılanmamış membran da sentezlenmiştir. Kitosan, polivinil alkol ve kitosan/polivinil alkol karışımlı hidrojel membranlarda uyumlu değildir. Membranların ısıl kararlılıkları bağlayıcı ajan olan glutaraldehit tarafından iyileştirilir. Sentezden sonra, reaksiyona girmemiş olan glutaraldehidin uzaklaştırılması için membranlar bir molar sodyum hidroksit çözeltisine daldırılıp ardından saf su ile yıkanmıştır. Tepkimeye girmeyen glutaraldehitin belirlenmesi için Tollen reaktifi kullanılarak nitel analiz uygulanmıştır. İyon baskılanmamış ve gümüş iyonları baskılanmış membranlar, FTIR, TGA/DTG, XRD ve SEM yöntemleriyle karakterize edilmiştir. FTIR analizi, koordine atomların polivinil alkoldeki hidroksil gruplarının kitosandaki amino grubuyla etkileşmesini göstermiştir. Gümüş iyonu baskılanmış membranlar iyon baskılanmamış membrane ve kitosan ile karşılaştırılmıştır. Gümüş iyonu baskılanmış membranlar etilendiamintetraasetik asit ve hidroklorik asitte hazırlanan tiyoüre çözeltisi ile rejenere edilmiştir. Membrandaki gümüş iyonlarının miktarı, atomik absorpsiyon spekroskopisi analiziyle belirlendi. Sonuç olarak, gümüş iyonlarının uzaklaştırılma kapasitesi etilendiamintetraasetik asit çözeltisine göre hidroklorik asitte hazırlanmış tiyoürede, daha yüksek olduğu bulunmuştur.

Anahtar Kelimeler: kitosan, iyon baskılanmış membrane, kitosan/PVA, gümüş uzaklaştırılması

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CHAPTER ONE

INTRODUCTION

1.1Chitosan

1.1.1 The Properties of Chitosan

Chitosan is a unique basic polysaccharide obtained by N-deacetylation (Dambies, Guimon, Yiacoumi, & Guibal, 2001; Sashiwa, 2005; Verma & Ray, 2005) of chitin in alkaline medium, which consists mainly of β-(1→4)-2-acetamido-2-deoxy-D glucose units and is the second most abundant biopolymer on Earth after cellulose, widely distributed in crustacean shells and cell walls of fungus (Dutta, Chattopadhyaya, & Tripathi, 2004; Mathur & Narang, 1990). Chitosan is a copolymer of N-acetyl D glucosamine and D glucosamine.

Chitosan becomes soluble in aqueous acidic media. The solubilization occurs by protonation of the NH_2 functional group on the C-2 position of the D-glucosamine repeating unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media.

The presence of NH_2 groups in chitosan is the reason why it exhibits much greater potential compared with chitin for use in different applications. It is a special biopolymer having good properties including biodegradability, biocompatibility, and antibacterial activity so it is interesting as a novel type of functional material. Chitosan is the only pseudo natural cationic polymer and thus has many applications in different fields (Kurita, 2001; Rinaudo, 2006)

Chitosan is commonly prepared by deacetylation of α-chitin using 40-50% aqueous alkali solution at 100-160°C for a few hours. The resulting chitosan has a degree of deacetylation (DA) up to 0.95.

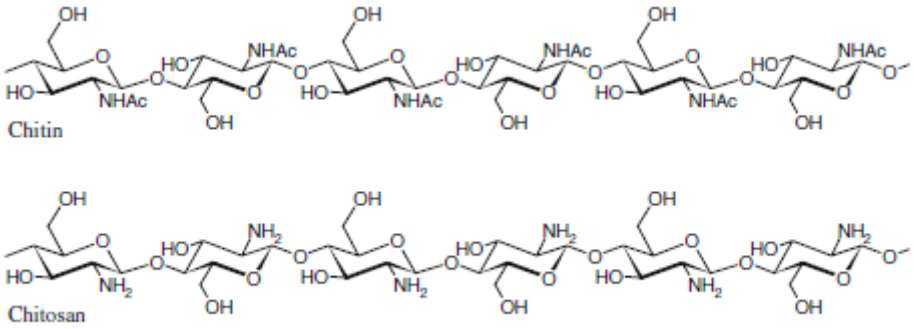
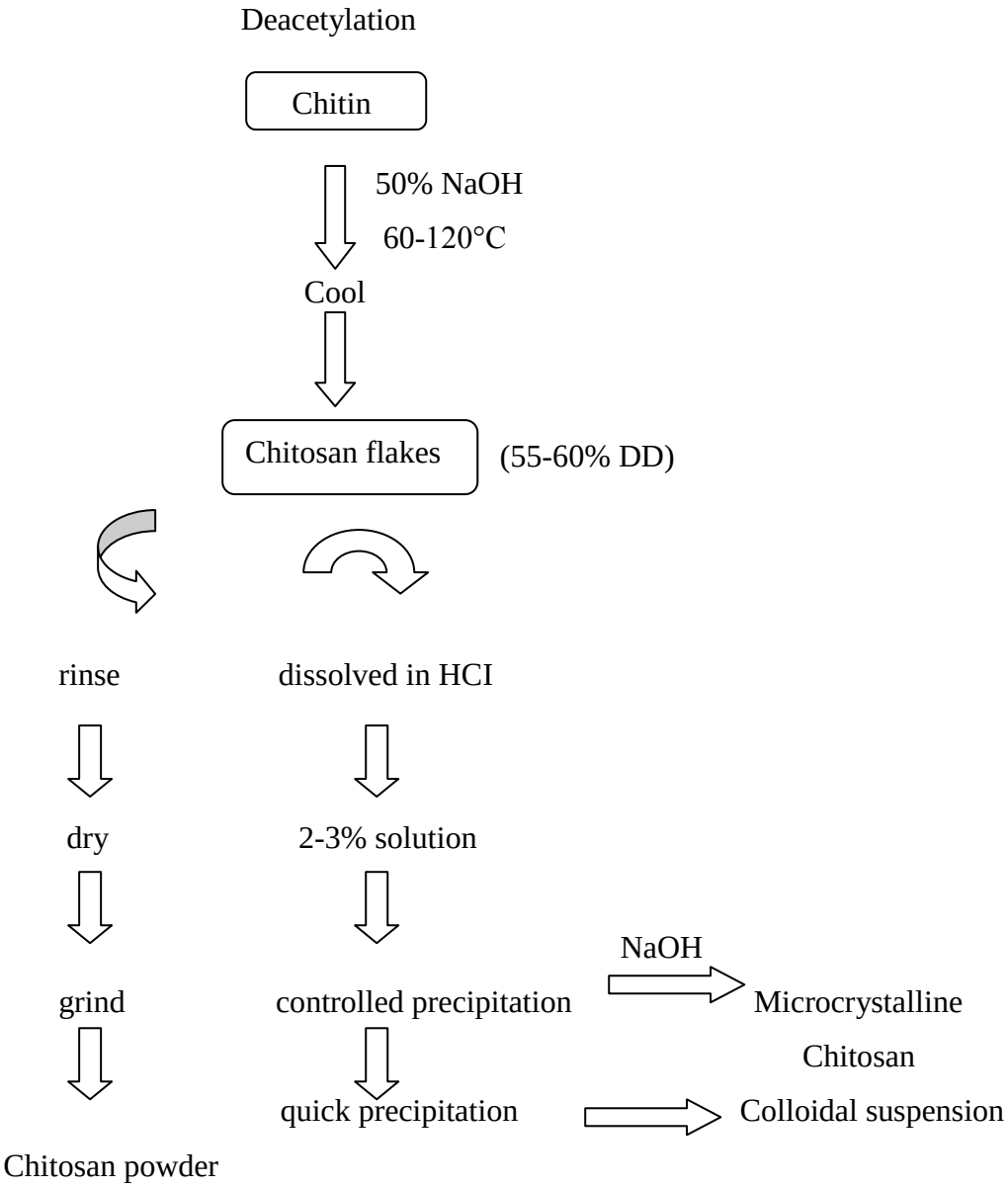


Figure1.1 Structures of chitin and chitosan



There are also problems in using chitin and chitosan. One of them is that it is difficult to dissolve in water and neutral pH range. However, when chemical modifications change the fundamental skeleton of chitin and chitosan, the modified chitin and chitosan lost the original physicochemical and biochemical activities. On the other hand, the chemical modifications of chitin and chitosan may have an advantage, because the modification with a hydrophilic reagent would be expected to result in hydrophilic chitin or chitosan while keeping the fundamental skeleton intact. Some approaches for the graft reaction of hydrophilic reagent onto chitin and chitosan were reported as a technique to improve the affinity to water or organic solvents (Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998).

The amino groups of chitosan are weak bases which are predominantly protonated when pH < 6.5, leading to the solubilization of the polymer only in acid dilute solutions. However, the poor solubility of chitosan above pH > 6.5 is a serious drawback in many of its potential applications.

It's a biocompatible, pH dependent cationic polymer, which is soluble in water up top pH = 6.2. Basification of chitosan aqueous solutions above this pH leads to the formation of a hydrated gel-like precipitate. Phase separation ensues from the neutralization of chitosan amine groups and the consequent elimination of repulsive interchain electrostatic forces, which subsequently allow for extensive hydrogen bonding and hydrophobic interactions between chains (Ruel-Gariepy, Chenite, Chaput, Guirguis, & Leroux, 2000).

Chitosan has many $–NH_2$ and –OH groups that can chelate heavy metal ions, providing high adsorption capacity and selectivity (Findon, McKay, & Blair, 1993).

There are numerous intermolecular and intramolecular hydrogen bonds in chitosan molecules, which strongly stabilize the packing structure of chitosan in the three unit cell directions (Lamarque, Viton, & Domard, 2004), and make chitosan have no melting point and only dissolves in some specific organic acids including formic, acetic, propionic, lactic, citric, and succinic acid, as well as in a very few

inorganic solvents, such as hydrochloric, phosphoric, and nitric acid (Wang, Turhan, & Gunasekaran, 2004).

Chitosan can also be prepared in a variety of forms, namely hydrogels and xerogels, powders, beads, films, tablets, capsules, microspheres, microparticles, nanofibrils, textile fibers, and inorganic composites. Chitosan is today a protagonist in advanced fields, for example it is a high performing non-viral vector for DNA and gene delivery (Weecharangsan, Opanasopit, Ngawhirunpat et al., 2008).

Biodegradable polymers such as chitosan need to be cross linked in order to modulate their general properties and to last long enough for delivering drugs over a desired period of time. Certain reagents have been used for cross linking chitosan such as glutaraldehyde, tripolyphosphate, ethylene glycol, diglycidyl ether and diisocyanate (Nishi, Nakajima, & Ikada, 1995; Speer, Chvapil, Eskelson, & Ulreich, 1980).

1.1.2 Applications of Chitosan

1.1.2.1 Biological Adhesive

Chitosan and its derivatives have been used in a wide range of applications. It has been found that the introducing of azide and lactose moieties into chitosan provides much better water solubility at neutral pH values. This type of chemical has been used as a biological adhesive for soft tissues. It is photo-cross-linkable by applying ultraviolet (UV) light, producing an insoluble hydrogel within 60 s. This material has great potential as a biological adhesive for medical use (Ono, Saito, Yura et al., 2000).

1.1.2.2 Antioxidant

Chitosan has antioxidant properties. Two types of fungal chitosan, B or C, have been prepared by alkaline N-deacetylation of crude chitin B or C for different durations of 60, 90, and 120 min (Yen, Tseng, Li, & Mau, 2007). Results show that chitosan has antioxidant activities of 61.6-82.4% at 1 mg cm^{-3} and shows reducing

powers of 0.42-0.57 at 10 mg cm^{-3}. Also, no significant difference in antioxidant properties between chitosan B and C has been observed.

1.1.2.3 Coatings, Biosensors, and Surface Conditioners

Chitosan may be used as a coating material. Recently, an electrophoretic deposition (EPD) method has been used for the fabrication of nanocomposite silica-chitosan coatings (Grandfield and Zhitomirsky, 2008). Good binding and film-forming properties of chitosan result in the formation of relatively thick coatings in the range of up to 100 μm. This process is achieved at room temperature, so the problems related to the high-temperature sintering of ceramic coatings on metallic substrates can be avoided.

Chitosan is also used as a coating material for fruits. The effects of edible chitosan coating on quality and shelf life of mango fruit has been studied (Chien, Sheu, & Yang, 2007). The results show that applying a chitosan coating effectively prolongs the quality and extends the shelf life of fruit.

1.1.2.4 Biomedical Applications

Chitosan and its derivatives are suitable for tissue engineering applications because of their porous structure, gel forming properties, ease of chemical modification, biodegradability, biocompatibility, antibacterial activity, and high affinity to in vivo macromolecules. It is one of the most important biomaterials in tissue engineering and shows considerably very good physicochemical and biological properties. Various types of chitosan derivatives have been used in skin, bone cartilage, liver, nerve, and blood vessel (Kim, Seo, Moon et al., 2008).

1.1.2.5 Antibacterial Properties and Food Packaging

Chitosan has been blended with nylon-6 by combining solvent evaporation and a phase-inversion technique, followed by chelation with silver ions. Gram-positive and Gram negative bacteria have been used to study the antibacterial properties of these membranes. The results show that the chitosan/nylon-6 blended membranes with Ag^{+}

are antibacterial against both Gram-positive and Gram-negative bacteria. By increasing the nylon-6 content, the antibacterial property of the blended membranes is decreased, which may be due to the corresponding decrease of chitosan and Ag^{+} content in the blend (Ma, Zhou, & Zhao, 2008).

A mixture of mint extract and chitosan was used as a new preservative for meat and meat products. Mint extract has good antioxidant activity but poor antimicrobial activity, while chitosan alone shows poor antioxidant activity with very good antimicrobial properties. The results indicate that 0.05% chitosan and mint mixture is the minimum inhibitory concentration and is more effective against Gram-positive bacteria. Therefore, use of mint and chitosan in meat products will improve their shelf life and safety (Kanatt, Chander, & Sharma, 2008).

1.1.2.6 Water Treatment

Recently, use of chitosan as an adsorbent has attracted attention in water treatment industries due to its high content of amino and hydroxyl functional groups. It shows high potential for adsorption of dyes, metal ions, and proteins. So it might be a good candidate for removing pollutants from water and wastewater.

Several treatment methods have been used for the removal of arsenic from water. In new research a biosorbent has been prepared for removing As (III) and As (V) from water by coating chitosan onto ceramic alumina (Boddu, Abburi, Talbott, Smith, & Haasch, 2008). The results show that this adsorbent has the potential to eliminate arsenic from drinking water and that it has more adsorption capacity for As (V) than for As (III). Sodium hydroxide (0.1 M) has been found to be effective in regenerating the column loaded with arsenic.

In another approach, heavy-metal ion sensors using chitosan-capped gold nanoparticles have been reported. The electrostatic attachment of chitosan onto gold nanoparticles has been considered for indicating the concentration of heavy-metal ions (Cu^{+2} and Zn^{+2}) in water (Sugunan, Thanachayanont, Dutta, & Hilborn, 2005).

1.1.2.7 Drug Delivery Systems

In recent years, significant effort has been devoted to the development of biodegradable materials for drug delivery systems. Among the various biodegradable polymers used for the development of controlled-release formulations, chitosan has been reported to be advantageous since it is a natural, nontoxic, biocompatible product with the potential for biodegradability.

A type of amphiphilic derivatives of chitosan has been synthesized by incorporating (2–hydroxypropyl-3-butoxy) propyl into succinyl-chitosan (Sui, Wang, Dong, & Chen, 2008). The results show that, by increasing the concentration, the surface tension decreases and aggregates form in solution. These chitosan derivatives may be used for controlled release of hydrophobic drugs.

1.1.2.8 Membranes

Chitosan has been widely used in membrane applications because of its high hydrophilicity, good film-forming character, and excellent chemical resistance properties. Several methods of membrane preparation may be used to improve separation performance, such as surface modification, blending, copolymerization, and grafting of a selective species onto an inert film. Chitosan-based membranes have been extensively studied not only for dehydration of some organic solvents such as alcohols, tetrahydrofuran (THF), isopropanol, and ethylene glycol, but also for the separation of organic/organic mixtures. It has also been used for pervaporation, ultrafiltration of biomaterials, and protein adsorption/separation.

Sodium alginate and chitosan have been used for preparation of enantioselective membranes (Kim, Jegal, &Lee, 2003). These materials have a high content of chiral active sites and very good hydrophilicity. It is important to mention that this high content of chiral active sites is crucial for the formation of a chiral environment in the membranes, which is critical for the separation of optical isomers. Since optical isomers show exactly the same chemical structure under achiral conditions, they cannot be distinguished. The membranes mentioned above are applied for optical resolution of α-amino acids, especially tryptophan and tyrosine. On increasing the

degree of cross-linking, these membranes achieve better enantioselectivity via increasing the interaction between the functional groups of the chiral environment of the membrane and the penetrating optical isomers.

1.1.2.9 Hydrogels

Hydrogels are three-dimensional networks that swell in water and aqueous solutions. It has physical properties similar to those of human tissues and possesses excellent tissue compatibility. The main disadvantage of hydrogels is their poor mechanical properties after swelling. In order to eliminate the disadvantage, hydrogels can be modified by physical blending (Amiji, 1995; Cascone, Sim, & Downes, 1995; Koyano, Koshizaki, Umehara, Nagura, & Minoura, 2000; Chuang, Young, Yao, & Chiu, 1999; Chandy & Sharma, 1992) or chemical modification by grafting (Yang, Chang, & Hsu, 1999; Yang, Wang, Hsu, Chang, & Lo, 1998; Yang; Huang, & Yeh, 1999; Yang, Jong, & Hsu, 1997) interpenetrating polymer networks (Peniche, Argulles-Monal, Davidenko et al., 1999; Gong, Zhang, Zhuang, & Lu, 1998) and cross linking method (Kushwaha, 1999; Ge, Cui, Yan, & Jiang, 2000). These materials, based on both natural and synthetic polymers, are currently attracting a great deal of interest as bioactive molecules and in tissue engineering. Among natural biopolymers of interest, chitosan stands out due to its unique combination of favorable properties. Chitosan hyrogels can be divided into two classes: physical and chemical. Chemical hydrogels are formed by irreversible covalent links, whereas physical hydrogels are formed by various reversible links. For various reasons, physically cross-linked hydrogels have attracted increasing attention as bioactive compounds.

Structure and interactions in covalently and ionically cross-linked chitosan hydrogels for biomedical applications have been critically analyzed (Berger, Reist, Mayer et al., 2004). It focused on chitosan hydrogels intended for medical or pharmaceutical applications. The properties of cross-linked hydrogels depend mainly on the cross-linking density, i.e., the ratio of moles of cross-linking agent to moles of polymer repeating units. The simplest structure of covalently cross-linked chitosan is self-cross-linked chitosan. Other structures are the hybrid polymer network, semi-

interpenetrating network, and ionic cross-linking of chitosan. Among these four types of chitosan hydrogels, covalently cross-linked hydrogels are the only systems characterized by a permanent network, due to their irreversible chemical links. Therefore, they exhibit good mechanical properties. Ionically cross-linked chitosan hydrogels exhibit greater swelling with pH changes compared with covalently cross-linked chitosan hydrogels.

1.2PVA

1.2.1 The Properties of PVA

Polyvinyl alcohols (PVA) are synthetic polymers used since the early 1930's in a wide range of industrial, commercial, medical and food applications including resins, lacquers, surgical threads and food-contact applications. General chemical and physical properties of PVA are summarized in Table 1.1

Table 1.1 General chemical identity and physical properties of polyvinyl alcohol

CAS No.	**9002-89-5**	**[a]USP/NF**
Molecular weight[*]	30000-200000	[b]Handbook Pharm. Excip.
Structural formula[*]	$(\text{-CH}_2\text{CHOH-})_n\text{-}(\text{-CH}_2\text{CHOCOCH}_3\text{-})_m$	[c]Japan Pharm. Excip. Dir.
Empirical formula[*]	$(C_2H_4O)_n(C_4H_6O_2)_m$	Japan Pharm. Excip. Dir.
Physical appeareance	Odorless, white to cream-colored granular powder	Handbook Pharm. Excip.
Specific gravity	1.19-1.31	Handbook Pharm. Excip.
Solubility	İnsoluble in aliphatic and aromatic hydrocarbons, ester, ketones, and oils; water soluble	Handbook Pharm. Excip.

[a] USP/NF, 2000. United States Pharmacopoeia (24) and National Formulary (19), pp. 1352-1353. U.S. Pharmacopeial Convention, Rockville, MD.
[b] Handbook of Pharmaceutical Excipients, 1994, second ed. A. Wade,P.J. Weller (Eds), pp. 383-384. American Pharmaceutical Association, Washington, DC.
[c] The Japanese Pharmaceutical Excipients Directory, 1996. Monograph on Polyvinyl Alcohol, p.355
[*] Variable based on PVA grade.

The physical characteristics of PVA are dependent on its method of preparation from the hydrolysis, or partial hydrolysis, of polyvinyl acetate (Fig 1.2). PVA is generally classified into two groups, partially hydrolyzed (A) and fully hydrolyzed (B).

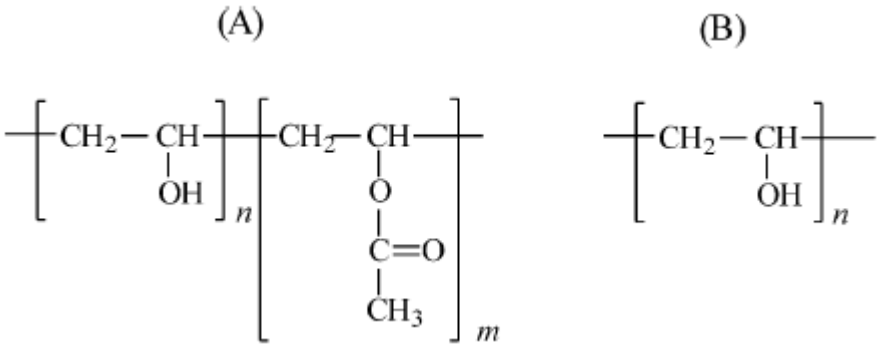


Figure 1.2 Structural formula for PVA: (A) partially hydrolyzed; (B) fully hydrolyzed

Varying the length of the initial vinyl acetate polymer and the degree of hydrolysis under alkaline or acidic conditions yields PVA products of differing molecular weights (20.000-400.000), solubility, flexibility, tensile strength and adhesiveness. Various properties are measured to characterize PVA such as pH, viscosity, loss on drying, melting point, refractive index, heavy metals and residue on ignition. These properties vary based on molecular weight and % hydrolysis for the grade of PVA.

1.2.2 Application of PVA

In the USA, majority of PVA is used in the textile industries as a sizing and finishing agent. PVA can also be incorporated into a water-soluble fabric in the manufacture of degradable protective apparel, laundry bags for hospitals, rags, sponges, sheets, covers, as well as physiological hygiene products.

PVA is also widely used in manufacture of paper products. As with textiles, PVA is applied as a sizing and coating agent. It provides stiffness to these products making it useful in tube winding, carton sealing and board lamination. PVA is used as a thickening agent for latex paint and common household white glue or in other adhesive mixtures such as remoistenable labels and seals, as well as gypsum-based cements such as is used for ceramic tiles. PVA is relatively insoluble in organic solvents and its solubility in aqueous solutions is adaptable to its necessary application. PVA can also be used for waste water treatment (Fao, 2004).

PVA has also been approved for use in packaging meat products by the Meat Inspection Division of the USDA and approved for use in packaging poultry products by the Poultry Division of the USDA.

PVA is approved for use in several medical applications including transdermal patches, the preparation of jellies that dry rapidly when applied to the skin and in immediate and sustained release tablet formulations. Cross-linked polyvinyl alcohol microspheres are also used for controlled release of oral drugs. Ophthalmic solutions, such as synthetic tears, may also contain PVA because it provides good dispersion and coating properties (Wade & Weller, 1994).

As an industrial and commercial product, PVA is valued for its solubility and biodegradability, which contributes to its very low environmental impact. Several microorganisms ubiquitous to artificial and natural environments such as septic systems, landfills, compost and soil have been identified which are able to degrade PVA through enzymatic processes. Combinations of oxidase and hydrolase enzyme activities degrade PVA into acetic acid but both the percent hydrolysis and its solubility affect the rate of PVA biodegradation (Fao, 2004).

1.3 Glutaraldehyde

1.3.1 General Definitions

Glutaraldehyde (CASRN 111-30-8) is an aliphatic dialdehyde whose major uses are based on its highly reactive chemical properties. Its ability to effectively cross-link cellular proteins imparts a wide array of biocidal activities. For this reason, glutaraldehyde is predominantly used as a general industrial antimicrobial in applications such as pulp and paper manufacture, water treatment, tanning, oil field, and other operations. It has been approved as an indirect food additive for various uses and is used in the medical field for cold sterilization of surgical instruments and endoscopes, X- ray film processing, and as a biological tissue fixative. It is commercially available as a 2% aqueous solution for hospitals uses, and as 25%, 45%, and 50% (v/v) aqueous solutions

Table1.2 Physicochemical properties of glutaraldehyde

Chemical name	Glutaraldehyde
Synonyms	1,5-Pentanedial; 1,2-diformylpropane; Glutaral dialdehyde; glutaric dialdehyde
CASRN	111-30-8
Molecular formula	$C_5H_8O_2$
Structural formula	$OHC-CH_2-CH_2-CH_2-CHO$
Physical state	Liquid
Odor description	Rotten apples
Molecular weight	100.13
Conversion factors	1 mg/m^3=0.24 ppm; 1ppm=4.1 mg/m^3
Boiling point	200.9 ^oC, with decomposition
Vapor pressure	0.102 Torr (50% solution); 0.003 Torr (2% solution) @ 209 ^oC
Vapor density (air=1.0)	3.4
Stability and reactivity	Incompatible with strong oxidizers and strong bases
Solubility	Soluble in water, alcohol, ether, and similar solvents

1.3.2 About Mechanism of Chitosan Cross-Linking with Glutaraldehyde

The reaction of cross-linking proteins by glutaraldehyde has been used over many years for enzyme immobilization to obtain different forms of heterogeneous biocatalyzers for pharmacological, medicotechnical, and biotechnological applications (De Santis & Jones, 1999; Jayakrishnan & Jameela, 1996; Migneault, Dartiguenave, Bertrand, & Waldron, 2004). At present, this reaction has again become the subject of great topical interest owing to the wide use of aminopolysaccharide chitosan, a unique biopolymer that possesses an instrict biological activity (antimicrobial, immunostimulating, and reparative capacity) (Kumar, 2000; Muzzarelli and Muzzarelli, 2005; Berger et al., 2004). The presence of a reactive amino group in the pyranose monomeric unit of chitosan makes it a candidate for the role of a carrier of biologically active compounds (Kurita, 2006).

The modification by bifunctional cross-linking reagents, of which GA is the most widely distributed, makes it possible to prepare, on the basis of chitosan, films, microcapsules, granules, fibers, hydrogels insoluble in water but possessing a high water-retaining capacity, and composite materials, and fix in their structure medicinal compounds, enzymes, and other proteins.

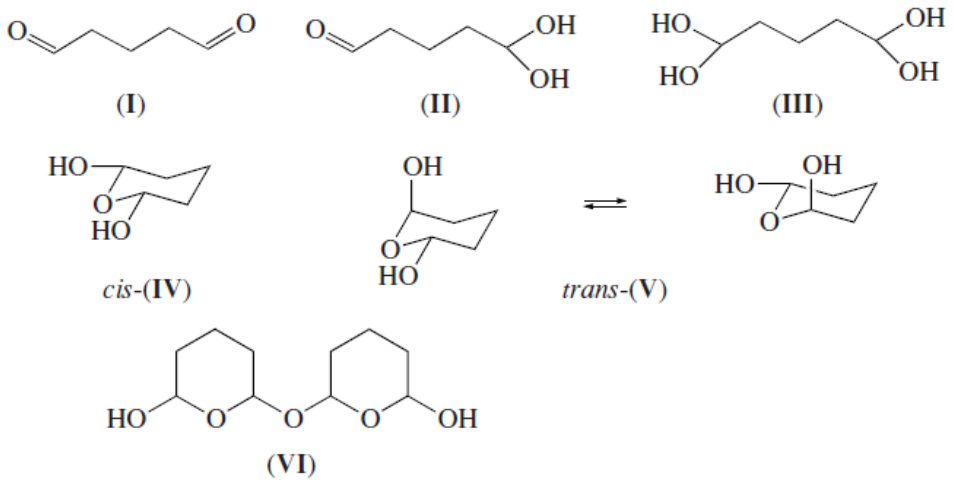


Figure 1.3 Structures of GA forms in aqueous solutions

A study of the NMR spectra of 25 and 50% GA solutions showed that GA exists in an equilibrium mixture of free aldehyde (I), mono (II) and dihydrate (III), as well as cyclic cis (IV) and trans (V) isomers (Figure 1.3). GA solutions also contain a polymeric form of GA (Kawahara, Ohmori, Ohkubo, Hattori, & Kawamura, 1992).

In an alkaline medium (pH 8-13), GA is polymerized, the polymerization rate increasing with the concentration of hydroxyl ions (Margel & Rembaum, 1980). Figure 1.4 shows the mechanism of the aldol reaction and aldol condensation. At the first stage, aldol (VII) is formed (aldol reaction), which is then dehydrated to an unsaturated derivative (VIII) (aldol condensation). Further condensation of products (VII) and (VIII) leads to polymeric products (IX) with molecular masses of 12 000-20000 (Margel & Rembaum, 1980).

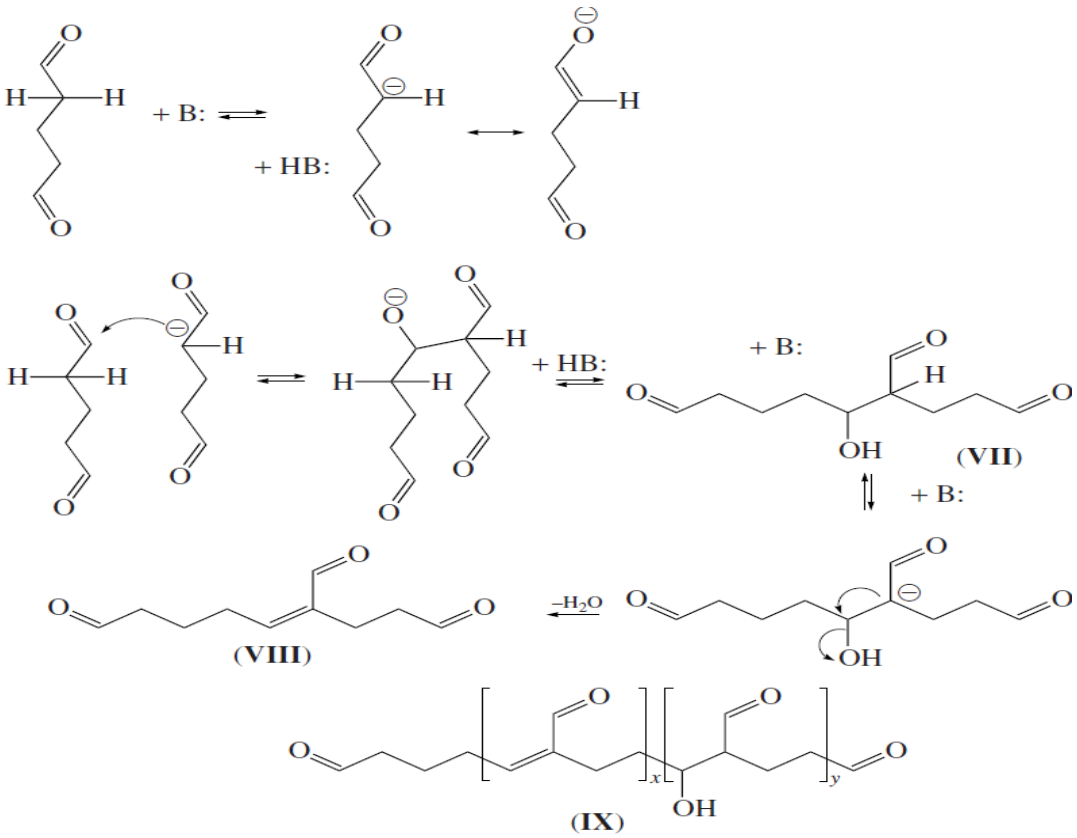


Figure 1.4 Mechanism of the aldol reaction and aldol condensation in alkaline medium.

If it is assumed that two carbonyl groups of GA are involved in the cross-linking by GA, then the theoretically possible modification of amino groups by a bifunctional cross-linking reagent should be achieved at the equimolar ratio of the aldehyde groups of GA and the amino groups of chitosan, which corresponds with regard for the deacetylation degree, to the GA/NH_2 ratio of 0.45 mol/mol. However, as the content of GA in the system increases above 0.4 mol/mol, the parameters characterizing the rigidity of the gel increase (Figure 1.5). These results also indicate that the reaction of the amino groups of chitosan with GA proceeds by a more complicated mechanism, which involves the formation of chitosan derivatives containing unsaturated oligomeric products of the aldol condensation of GA (IX) capable of reacting further not only with each other but also with the amino groups of chitosan after the completion of gel formation.

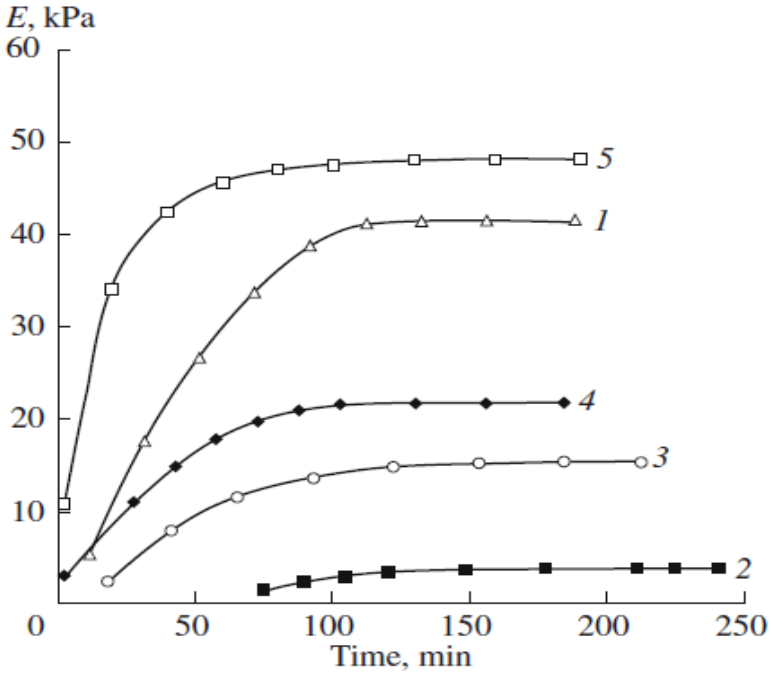


Figure 1.5 Kinetics of changes in the shear module for gels of chitosan cross-linked by GA. The GA/NH_2 ratio 2.5 (*1*), 1.0 (*2*, *3*, *5*), 0.4 (*4*), and pH 4.1 (*1*–*3*) and 5.6 (*4*, *5*). The chitosan concentration in the reaction mixture is 1.31 (*1*, *2*, *4*, *5*) and 1.9% (*3*).

The formation of the cross-linked product can occur through the condensation of the amino groups of chitosan with the carbonyl groups of modified chitosan, or through the aldol reaction and condensation of oligomeric chains of modified chitosan with the formation of the products shown in Figure 1.6.

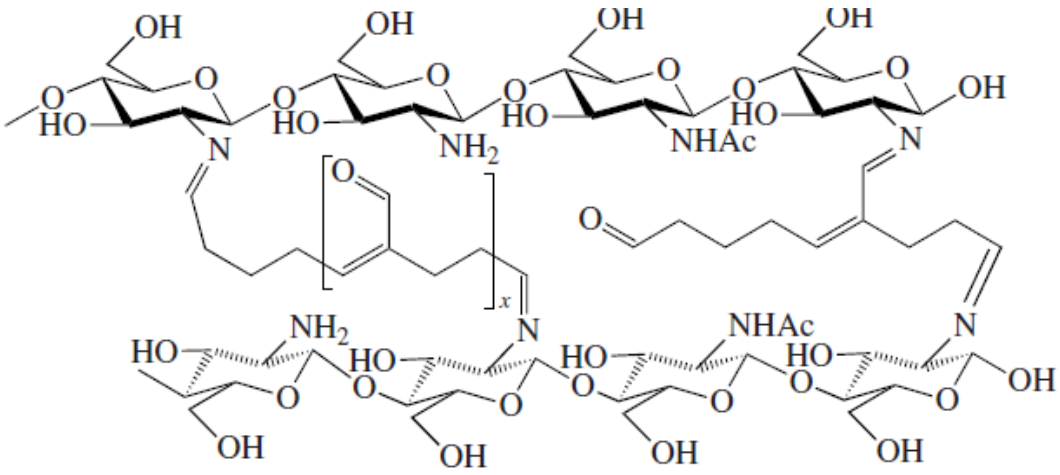


Figure 1.6 Structures of the products of reaction of chitosan and GA

The cross-linking of chitosan by GA is a complex process: chitosan catalyzes the polymerization of GA to form irregular products (Figure 1.6), with the length of oligomeric chains in modified or cross-linked chitosan and the concentration of coupled bonds N=CHCH=C< and O=CHCH=C< being dependent on the conditions under which the process is conducted (the concentration of GA and pH of the reaction medium). This fact should be taken into consideration if the reaction of chitosan with GA is used to create materials for medical or medico biological applications.

1.4 Molecular Imprinting

Molecular recognition is a phenomenon that can be envisaged as the preferential binding of a molecule to a receptor with high selectivity over closely related structural analogues. This concept has been translated elegantly into the technology of molecular imprinting, which allows specific recognition sites to be formed in synthetic polymers through the use of various templates. Currently two fundamental approaches to molecular imprinting may be distinguished. One of these is covalent or preorganized approach mainly developed by Wulff and Sarhan (Wulff and Sarhan, 1972), where the template- monomer construct in solution prior to polymerization is maintained by reversible covalent bonds and the recognition of the template is dependent on the formation and cleavage of these bonds. The other major type is the non-covalent imprinting or self-assembly approach, advocated mainly by Mosbach and co-workers (Arshady & Mosbach, 1981), where the prearrangement between the template and monomer(s) is formed by non-covalent interactions and subsequent recognition is also dependent on these interactions. In paralel with these strategies, another method semi-covalent approach termed as sacrificial spacer methodology has been introduced (Whitcombe, Rodriguez, Villar, & Vulfson, 1995), which takes the advantage of a combination of the above approaches, with strong covalent bonds being used in the imprinting step, and non-covalent interactions used in the recognition process after cleavage of the template from the polymer.

Ion-imprinted polymers (IIPs) are similar to MIIPs, but they recognize metal ions after imprinting, while retaining all the virtues of MIPs.

Molecular imprinting has high potential to provide an additional level of control in the rational engineering of drug delivery matrices. Further control of therapeutic transport through the macromolecular structure as well as optimizing the number of therapeutic molecules to polymer chains are leading to significant enhancements in drug loading and extended transport. This level of control has only been demonstrated recently within a small number of research articles and is expected to grow significantly in the future. This will be particularly important in reduced length scale applications with micro and nanoscale carriers and devices.

1.5 Metal Ion Imprinted Membrane

Selective enrichment and separation of metal ions is an important research field. These metallic ions mainly include toxic heavy metal ions in water (Chen, Yang, et al., 2009; Li, Su, & Tan, 2008), low content of rare metal ions in the earth crust as well as some precious metals. Owing to its excellent properties of electrical and thermal conductivity and ductility, silver has been widely applied in fields of aerospace, communications, chemical industry, medical equipment, electroplating, photographic materials, electronic industries, etc. As known, however, silver resources in the world are extremely scant and mainly associated with lead, copper and antimony deposits. In addition, there are considerable waste solutions, containing silver ions, produced by electroplating wastewater, waste fixative as well as the Ag-containing wastewater in laboratory. Therefore, silver recovery from waste solutions becomes more and more important with developing society. However, due to the low concentration, it is necessary to enrich and separate silver ions before recovery.

There are some traditional techniques to recover silver from waste solutions such as electrolysis, replacement, precipitation and ion exchange in industry. Recently, (Donia, Atia, & Elwakeel, 2007) have studied a chemically modified chitosan with magnetic properties, which can be used in silver recovery from aqueous solutions. The membrane has wide application in metal enrichment and separation, and membrane technology is an established part of several industrial processes (Nunes & Peinemann, 2001). While, the common membrane does not perform very well on selective enrichment and separation of the target metal ions, and the problem can be

effectively solved by the technique of molecular imprinting, which is a convenient and powerful technique recognizing the imprinted molecule selectively by preparing polymeric materials with artificial receptor-like binding sites for various substances (Xu, Zhu, & Chen, 2004; Yin, Yang, Chen, 2005; Liu, Xu, Yan, & Gao, 2005; Guo, Xia, Wang, Song, & Zhang, 2005).

Molecularly imprinted polymers represent a new class of materials possessing high selectivity and affinity for the target molecule. Molecular imprinting involves arranging of functional groups around a template molecule, subsequent polymerization results in trapping template molecules in highly cross linked polymer matrix (Khajeh, Yamini, Ghasemi, Fasihi, &Shamsipur, 2007). Extraction of the imprint molecules from matrix leaves predetermined arrangement of ligands and a tailored-binding pocket. Three steps are involved in ion-imprinting process:

1. Complexation of metal ion (template) to polymerizable ligand,
2. Polymerization of this complex,
3. Removal of metal ion after copolymerization.

After removal of the target ion, the prepared polymer is put into a solution containing metal ions from which the imprinted ion should thus be preferentially extracted.

Nishide et al. have utilized for the first time ion template effect in the synthesis of chelating polymers way back in 1976 (Nishide, Deguchi, & Tsuchida, 1976).They cross linked a linear chain polymer, poly (4-vinylpyridine) with a bifunctional reagent (dibromoalkane) in the presence of metal ions. Takagi and his colleagues introduced the concept of surface imprinting polymers, wherein ion imprinted polymer (IIP) particles were prepared by emulsion polymerization involving a functional host monomer, an emulsion stabilizer, a cross linking agent, and a metal cation template, which was selectively complexed by the metal binding groups at the aqueous-organic interface to form recognition sites (Yu, Tsukaghoshi Maeda, & Takagi, 1992; Koide, Shoshenji, Maeda, & Takagi, 1998). Subsequently, the

template cation was removed by acid stripping. An alternative approach was based on the copolymerization of isolated or nonisolated monomer/ion complexes with the cross linking agents. Using this approach, IIP's were developed with different inorganic ions as imprint ions (Bae, Southard, & Murray, 1999; Vigneau, Pinel, & Lemaire, 2001; Vigneau et al., 2002; Biju, Gladis, & Prasada Rao, 2003).

Different approaches for the synthesis of IIPs were reviewed by (Prasada Rao, Kala, & Daniel, 2006). Four mechanisms may be established;

1. Cross-linking of bi-functional reagents with linear chain polymers,
2. Chemical immobilization of vinylated ligands in the polymer matrix,
3. Surface imprinting by emulsion polymerization,
4. Trapping a nonvinylated chelating agent inside the polymer matrix.

Ion imprinted materials have been used in various fiels, including chromatography (Ersöz, Say, & Denizli, 2004), sensors (Metilda, Prasad, Kala, et al., 2007), and solid phase extraction (Prasada Rao, Sobhi Daniel, & Gladis, 2004).

1.5.1 Related studies

Kimaro et al. reported the preparation of uranyl ion permselective membrane via bulk polymerization of uranyl vinyl benzoate and styrene/DVB after addition of 2-nitrooctylphenyl ether (plasticizer) and polyester prepared from diglycollic acid and 1,6-hexanediol. Competitive transport experiments exhibited better selectivity for uranyl ion over other bivalent transition metal ions (Kimaro, Kelly, & Murray, 2001). Araki et al. prepared zinc (II) ion-selective membrane by surface molecular imprinting technique utilizing water-in-oil (W/O) emulsion polymerization. To obtain flexible and mechanically stable membranes for practical applications, the polymerization was conducted in the presence of acetonitrile-butadiene rubber and hydrophilized poly (tetrafluoroethylene) membranes. The imprinted membrane showed higher adsorption affinity and permeation selectivity towards the imprinted zinc ion than the non-imprinted counter part of Cu^{2+}. The obtained results indicated the permeation mechanism of metal ions as hopping on binding sites of the

membranes (Araki, Maruyama, Kamiya, & Goto, 2005). Murray described the MIP-based membrane prepared by copolymerizing a matrix monomer, cross-linking agent, ion-imprinting complex, permeability agent and polymerization initiator for the removal of phosphate, nitrate and ferric ions (Murray, 2005).

Zhai et al. reported to preparation of zinc-small molecule complex imprinted membrane using PVDF (0.45 μm) as the supporting material, in which 2, 2'-bipyridyl ligand was added to Zn (II) water solution to form complex. This complex was adsorbed or rejected by the prepared membranes. The relative selectivity factor of the imprinted membrane for the target ion of Zn (II) versus Cu (II) was 2.12 (Zhai, Liu, Chang, Ruan, & Liu, 2008).

The metal ion imprinted membrane using blends of chitosan and polyvinyl alcohol as film-forming materials with silver ions as template was prepared by Wang et al (Wang, Zhang, Ma, et al., 2009). Finally, Shawky reported the synthesis of a novel ion–imprinted membrane for selective removal of Ag (I) ion from aqueous solution. The membranes were obtained via cross-linking of chitosan (CS), PVA, and blended chitosan/PVA using glutaraldehyde (GA) as cross-linker (Shawky, 2009).

A Ni (II) imprinted membrane was successfully prepared by polymerization of functional and cross-linking monomers around a template Ni (II) dithizone molecule. The resulting membrane exhibited high adsorption capability towards the template, namely Ni^{2+} using dithizone as a ligand. In permeation studies using the Ni (II) imprinted membrane, selective permeation of Ni^{2+} versus Co^{2+} was observed. This suggests that the sorption of metal ions is initially the rate-determining step in the membrane permeation. After fifth cycle of adsorption/desorption process, the adsorption capacity of the recycled imprinted membrane was maintained at around 90% of the original value (Vatanpour, Madaeni, Zinadini, & Rajabi, 2011).

An interpenetration network (IPN) ion-imprinting hydrogel (IIH) was syntesized using uranyl ions as template for adsorption and removal of uranyl ions from aqueous solutions. The IIH was obtained via cross linking of blended

chitosan/polyvinyl alcohol (PVA) using ethylene glycol diglycidy ether (EDGE). The ability of the IIH to adsorb and remove uranyl ions from aqueous solutions was assessed using a batch adsorption technique. The selectivity coefficient of uranyl ion and other metal cations on IIH indicated an overall preference for uranyl ions which was much higher compared with the non-imprinted hydrogel (Liu et al., 2010).

1.5.2 Ion-Imprinting Chitosan/PVA Crosslinked Membrane

CS membranes have been extensively prepared and used for different purposes (Xiao, Feng, & Huang, 2007). The use of CS membranes as an adsorbent also provides the potential for regeneration after adsorption and reuse of the membranes in subsequent adsorption operations. However, these membranes have the disadvantage of poor chemical resistance and mechanical strength. This disadvantage significantly reduces the recycle life of the chitosan beads.

On the other hand, poly (vinyl alcohol) (PVA) is well known as a membrane-forming material with high hydrophilicity, good chemical resistance, and mechanical property.

Glutaraldehyde (GA) is a common cross linker used in polypeptide and protein cross linking because of the high activity of the aldehyde groups, which readily form Schiff's base with amino groups of proteins. GA is also used as a cosslinking agent for PVA (Wang et al., 2004) and CS (Ruiz, Sastre, & Guibal, 2004).

CS/PVA combination in the presence of GA has shown promising attributes for practical application, such as high mechanical strength, high swelling and shrinking ratio, high pH sensitivity, and biodegradability. However, the adsorption capacity for heavy metal ions for cross linked CS was lower when compared with free CS, because of functional groups ($-NH_2$) being cross linked.

Ag (I) IIM has large adsorption capacity to Ag (I). During the process of imprinting, metal complexing plays the main role and the coordinating atoms may be N atom of $–NH_2$ in CTS and O atom of -OH in PVA (Wang et al., 2009).

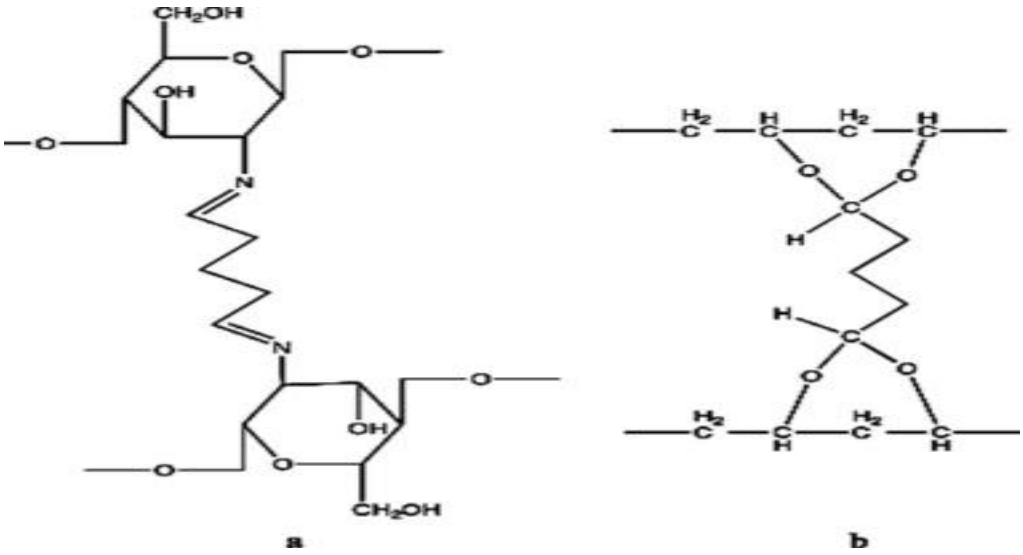


Figure 1.7 Schematic representation of (a) chitosan and (b) PVA crosslinked with glutaraldehyde.

1.6 Purpose of the Study

The presence of soluble silver in wastewater is a problem shared by many industrial processes. The two major sources are the photographic and electroplating industries, but mining, silver recovery plants, and the medical technology industry are also contributors. Local regulations limit the amount of silver that may be present in wastewater and classifies silver as a hazardous substance. Noteworthy to maintain that silver is a valuable metal, several recovery methods, primarily electrodeposition and metallic replacement, have had a history of use before implementation of wastewater regulations. Precipitation and ion-exchange are two additional methods currently used to remove silver from wastewater. All these methods have associated problems. Electrodeposition is expensive and does not easily remove silver at low concentrations. Metallic replacement is cheaper, but it only removes ~95% of the silver from solution, and the treated wastewater can still be above the legal limit. Furthermore, the product of both these techniques is a sludge that is expensive to refine. Precipitation works well to reduce silver concentration to low levels but requires the use of additional chemicals to perform the task, and the resulting precipitates are also impure sludges that require further treatment. The ion-exchange method is effective at any silver ion concentration but is expensive to install and maintain.

One of the new developments in recent years to accumulate precious metals or remove toxic metals from dilute solutions is the use of adsorbents of biological origin, including alginate, dead and living biomass, chitosan, lignin, carrageenan, and so on. Chitosan (CS) is produced from N- deacetylation of chitin, a major component of crustacean shells and fungal biomass, and is readily available from sea-food processing wastes. Chitosan has demonstrated the potential to adsorb significant amounts of metal ions. However, chitosan-based adsorbents have weak mechanical properties and poor mechanical properties and poor chemical resistances. For these reasons, much effort has been focused on the interpenetration network (IPN), prepared by introduction of a second hydrophilic polymer into the chitosan matrix, because of its high hydrophilicity, good chemical resistance and mechanical property. CS membranes have been extensively prepared and used for different purposes. The use of CS membranes as adsorbent also provides the potential for regeneration after adsorption and reuse of the membranes in subsequent adsorption operations. However, these membranes have the disadvantage of poor chemical resistance and mechanical strength.

In this study, ion-imprinted polymer membrane for selective removal and preconcentration for Ag (I) ions from aqueous solutions and CS/PVA blended hydrogel membranes will be synthesized. The membranes will be obtained by cross linking of chitosan/PVA blend. In the synthesis, glutaraldehyde (GA) will be used as a cross linker. The microstructure, morphology and crystallinity of the blended samples will be characterized through X-ray diffraction (XRD), scanning electron microscopy (SEM) and FTIR (Fourier transform infrared spectroscopy) analysis. Thermal properties of the membranes will be also determined by TGA. Quality analysis will be applied on the samples by using Tollen reactive for the determination of the unreacted GA. The amounts of Ag-ions will be determined by the atomic absorption spectroscopic analysis.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

Chitosan (CS) (highly viscous) was purchased from Fluka (degree of deacetylation: 75-85%, average molecular weight: 500000-700000 g mol^{-1}) as a flaked material. Acetic acid (HAc) and sodium hydroxide (NaOH) were obtained from Riedel de Haen. PVA was purchased from Fluka (average molecular weight 72 000). Glutaraldehyde (GA) was purchased from Aldrich Chemical (25% (wt %) aqueous solution).

2.2 Preparation of Membranes and Solutions

2.2.1 Chitosan and PVA Solution Preparation

PVA hydrogel was prepared by fully dissolving 0.1490 g polymer powder without further purification in 3 mL of distilled water, under magnetic stirring at 75°C (5% PVA) about 2 hours. PVA 5% solution was left to cool down to room temperature and the pH was corrected to (2.00±0.05) with 1 M HCI.

1 g Chitosan was dissolved in 100 mL of distilled water with 2% of CH_3COOH, under magnetic stirring at 1400 rpm, about 26 hours.

2.2.2 Preparation of Chitosan/PVA Blended Hydrogel Membrane

5% PVA solution was added into the 1% chitosan solution to obtain chitosan/PVA molar ratio of (3:1). The mixture was aged under stirring for 24 hours until the PVA and chitosan completely formed a clear solution. According to the ratio of $\frac{n_{GA}}{n_{NH_2}} = 0.42$; 3.92 mL of 5% glutaraldehyde (the cross linker reagent) was slowly added under constant stirring for 1 hour. Further, in the sequence, the solution was poured into plastic moulds. It was dried for 16 hours in vacuum drier.

2.2.3 Preparation of Ion-Imprinting Membrane

0.75 g chitosan was dissolved in 75 mL of distilled water with 2% CH_3COOH under magnetic stirring at 1400 rpm about 26 hours. PVA hydrogel was prepared by fully dissolving 0.1116 g of polymer powder without further purification in 2.23 mL of distilled water, under magnetic stirring at 75°C (5% PVA) about 3 hours. 5% PVA solution was left to cool down to room temperature and the pH was corrected to (2.00 ± 0.05) with 1 M HCI. After that, 5% PVA was added into the 1% chitosan solution to obtain chitosan/PVA molar ratio of (3:1). The mixture was kept under stirring for 24 hours until PVA and chitosan completely formed a clear solution. Then, 0.93 mL of 0.01 M $AgNO_3$ was added into this mixture, and chitosan, PVA and $AgNO_3$ was stirred about 2.5 hours. After that, according to the ratio of $\frac{n_{GA}}{n_{NH_2}} = 0.42$; 2.94 mL of 5% glutaraldehyde was slowly added under constant stirring for 1 hour. Further, the solution was prepared into Petri dish and allowed to dry for 72 hours at room temperature, and then in oven at 40°C for 48 hours. An individual ion-imprinted chitosan and PVA membranes were also obtained. After that, membranes were immersed in 1 M NaOH for 1 day, followed by repeatability washing with de-ionized water to eliminate any unreacted GA.

Finally, the Ag(I) imprinted in the membrane was removed by treating with EDTA and 0.5 M thiourea in 1M HCl in a shaker bath 48 hours. The silver released was determined by FAAS.

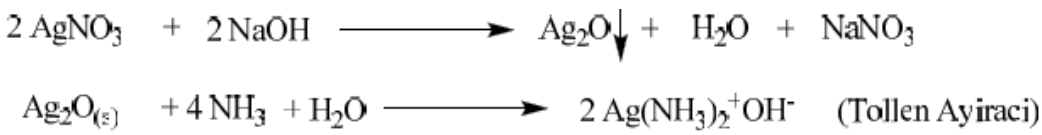
2.2.4 Desorption Studies for Ag

Desorption of Ag^+ ions were studied with 0.01 M EDTA solution and 0.5 M thiourea in 1 M HCI solution. The membranes were placed in these desorption medium and stirred continuously at 25°C 80 min^{-1} in shaker bath for 48 hours. The final Ag^+ concentration in aqueous phase was determined by FAAS

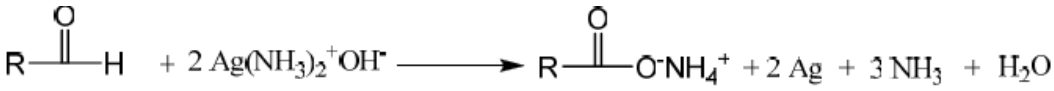
2.2.5 Removal of Unreacted Glutaraldehyde

The release of GA from cross linked membranes were evaluated by means of a quality analysis with Tollen test.

Tollen reagent which silver oxide or silver nitrate is solved in ammonia is ammonia-silver ion solution



While tollen reagent is reduced to metallic oxide by aldehyde, aldehyde is oxidized to acid. Ketones aren't usually oxidized by tollen reagent.



Tollen test is positive when metallic silver like bright mirror appearance collects on the surface of test tube. If test tube is not clean enough, silver metal will accumulate as black residue.

Attention: Tollen reagent must prepare freshly.

Test tube must be clean. 0.5 mL of 5% NaOH is added into 2 mL of 5% $AgNO_3$, and is stirred. Until precipitation is solved, 2% NH_3 solution is added by drop wise. (If NH_3 solution is added excessively, test is negative. So it must be prepared carefully).

0.5 mL of sample is added into test tube. Then 1 mL Tollen reagent is added, too. Test tube is stirred and is waited about ten minutes. If there is no change, test tube is heated at 30 or 35°C about 5 minutes.

2.3 Characterization

2.3.1 Fourier Transform Infrared (FTIR) Spectra of The Samples

FTIR was used to characterize the presence of specific chemical groups in the hydrogels and investigating the formation of cross linked networks from the blends with glutaraldehyde.

Fourier transform infrared (FTIR) spectra (absorbance) were measured on a Pelkin-Elmer FTIR spectrophotometer Spectrum BX-II in the range 4000-400 cm^{-1} at a resolution of 4 $cm^{-1.}$

2.3.2 Thermal Analysis

The thermal properties of the samples were investigated by thermogravimetric analysis (TGA). TGA was performed at 600˚C at a rate of 20˚C/min with a Perkin Elmer Diamond TG/DTA instrument. The mass of samples varied from 2 to 3 mg.

2.3.3 Scanning Electron Microscopy (SEM)

The morphology of the films obtained was assessed by scanning electron microscopy (SEM). The images were obtained using an accelerating voltage of 10 kV. Before examination the samples were sprayed with a fine layer of gold using a low deposition rate, refrigerated and placed at the maximum distance from the target to prevent damage to them. SEM photographs were taken at different magnifications (in the range of 50X and 5000X) by using Jeol JSM 60 model SEM apparatus equipped with energy dispersive X-Ray (EDX) in Metallurgy and Materials Engineering Department of Dokuz Eylül University/Izmir.

2.3.4 Crystallinity by X-Ray Diffraction (XRD)

X-ray diffraction (XRD) patterns of pure chitosan, chitosan/PVA blend with glutaraldehyde, Ag (I) ion-imprinted chitosan and PVA membranes were recorded on X-ray diffractometer The scanning rate was 5°/min and scanning scope of 2θ was range from 0° to 60° at room temperature.

2.3.5 Flame Atomic Absorption Spectrometer (FAAS)

Ag^+ concentrations were determined using Perkin Elmer AAnalyst 700 model flame atomic absorption spectrometer with deuterium background correction. The radiation sources were hollow cathode lamps. The instrumental conditions were selected as suggested by manufacturer and given below. The acetylene flow rate and the burner height were adjusted in order to obtain the maximum absorbance signal.

Table 2.1 Instrumental parameters for metal determination by FAAS

Element	**Wavelength (nm)**	**Slit width (nm)**
Ag^+	338.3	0.7

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared (FTIR) Spectra of the Samples

FTIR spectroscopy was used to assess the polymer chemical groups (chitosan and PVA) and investigating the formation of cross linked networks from the blends with glutaraldehyde. Figure 3.1 a - c shows the FTIR spectra of CS/PVA/GA blend, b shows the spectra of Ag imprinted membrane. The spectrum of chitosan shows the characteristic absorption bands at 1650 cm^{-1} (amide I), 1556 cm^{-1} (amide II), and 1402 cm^{-1} (-CH_2 bending). The absorption bands at 1140 cm^{-1} (anti-symetric stretching of the C-O-C bridge), 910 and 1063 cm^{-1} (skeletal vibrations involving the C-O stretching) are characteristics of its saccharide structure (Yin et al., 1999; Traravel & Domard, 1993). The broad band at 3349 cm^{-1} is caused by amine N-H symmetrical

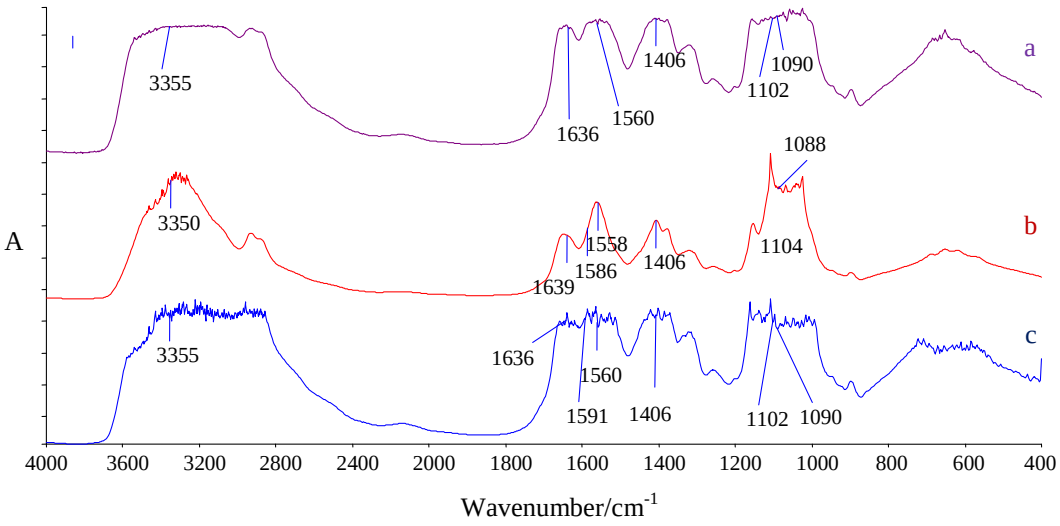


Figure 3.1 FT-IR spectra of (a) CS/PVA/GA2, (b) CS/PVA/GA/Ag, (c) CS/PVA/GA1

According to FT-IR spectra, the shapes and positions of most bands are exhibited similarly. Figure 3.1a and c shows FT-IR spectra of CS/PVA blend with different concentrations of GA chemical cross linker, 13.5% (curve c) and 17.5% (curve a) It can be noted the band at 1110, 1406, 1638 and 1650 cm^{-1} mainly associated with PVA, and also the presence of bands related to carboxylic acid and the imines

formed by the cross linking reaction by glutaraldehyde of amine groups from chitosan. Moreover, an increase in the intensity and a shift in the band associated with the bending vibration of the CH_2 (1406 cm^{-1}) group was observed. As expected, because the blend cross linking reaction was conducted at pH=4.0, covalent chemical bonds have preferentially occurred in the chitosan amine groups and less in the PVA hydroxyl groups (Brugnerotto, Lizardi, Goycoolea et al., 2001; Costa & Mansur, 2007; Mansur & Costa, 2008; Rao, Naidu,Subha, Sairam, & Aminabhavi, 2006; Shigemasa, Matsuura, Sashiwa, & Saimoto, 1996; Wang et al., 2004a, 2004b). Chemical cross linking of the chitosan/PVA blends can be explained by the Schiff base formation as verified by the 1634 and 1550 cm^{-1} bands associated with the C=N and NH_2 groups, respectively (Costa & Mansur, 2008; Rokhade, Patil, & Aminabhavi, 2007; Wang et al., 2004a, 2004b). All chitosan-derived blends have shown a relative increase on their imine (-C=N-) band at 1634 cm^{-1} and simultaneous drop on the amine (-NH_2) band after chemical cross linking with glutaraldehyde. The imine group was formed by the nucleophilic reaction of the amine from chitosan with the aldehyde.

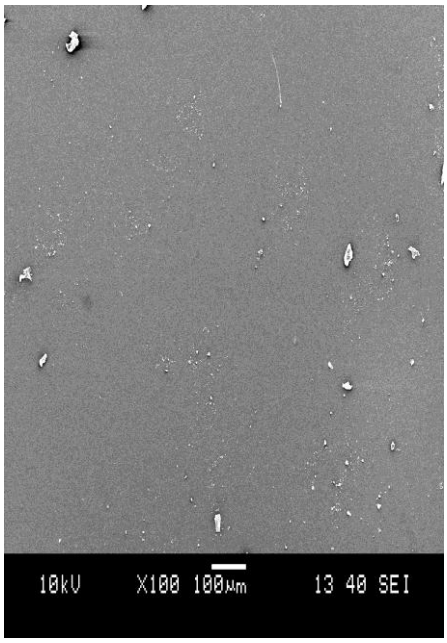
Compared with b and c, the graphics show that the band at 1090 cm^{-1} assigning to C-O stretching vibration of PVA after blending (Figure 3.1 c) moves to 1088 cm^{-1} (Figure 3.1 b) when imprinted ions existed, which indicates that O atom of –OH in PVA participates in the coordination reaction. –NH_2 and – OH of chitosan stretching vibration couple and form abroad band around 3356 cm^{-1} after blending in Figure3.1.c while the corresponding band moves to 3350 cm^{-1} in Figure3.1 b which indicate that –NH_2 or –OH of chitosan participates in the complexing with Ag (I) and form coordination compound. The absorption band around 1589 cm^{-1} corresponding to –NH_2 deformation vibration of chitosan after blending (Figure3.1 c) shifts to low frequency of 1587 cm^{-1} in Figure3.1 b. Therefore it can be thought that the coordinating atom is not O atom of –OH but N atom of –NH_2 of chitosan, which in accordance with Chunmei Ding's research (Ding, Chen, Li, Zhang, & Qiao 2005). To sum up, it can be predicted that N atom of –NH_2 of chitosan and O atom of –OH in PVA involve the coordination reaction with Ag (I).

3.2 Scanning Electron Microscopy Analysis (SEM)

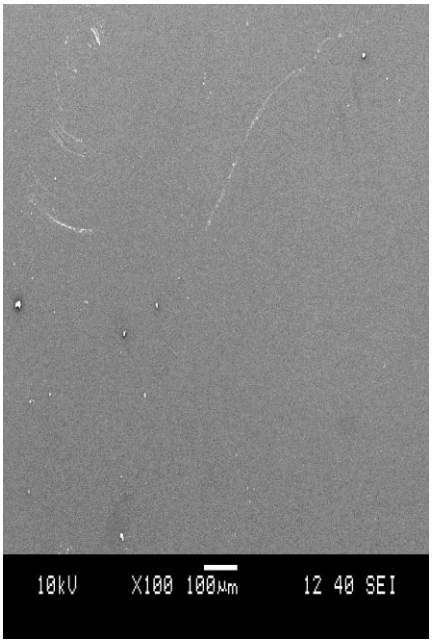
In order to investigate difference between non-imprinted and Ag imprinted membrane, surface morphology of membranes were studied by using SEM analysis. SEM results were presented in Figure 3.2 which had non imprinted membrane at different glutaraldehyde concentration and Ag imprinted membrane.

Non imprinted membranes at different glutaraldehyde concentration have very similar morphology. But, at higher and lower glutaraldehyde concentration, there were some differences. At higher concentration, it was shown that there were fewer holes in the membrane. Chitosan and PVA formed a chemically bonded hydrogel with glutaraldehyde since glutaraldehyde has the aldehyde groups, which readily form Schiff's base with amino groups of chitosan. However, at lower glutaraldehyde concentration, PVA and chitosan may not be cross linked by glutaraldehyde. They had their chains mostly physically entangled in the hydrogel network. On the other hand, Ag imprinted membrane had different morphology when compared with non-imprinted membranes. The reason is that Ag ions were allowed to enter these holes and the imprinted ion was uniformly coated on the surface of membrane.

As a result, Ag imprinted membrane was coated the surface of membrane, whereas non-imprinted membranes had holes.



A



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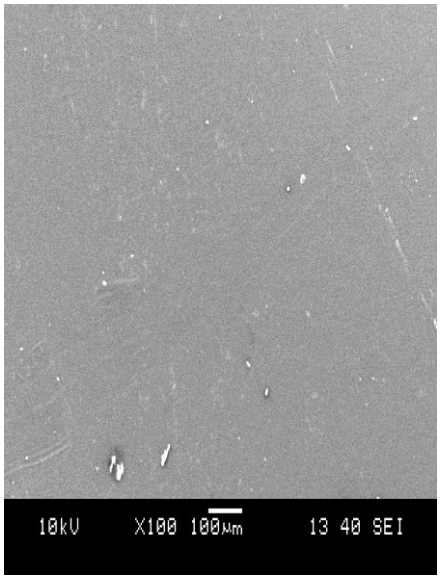
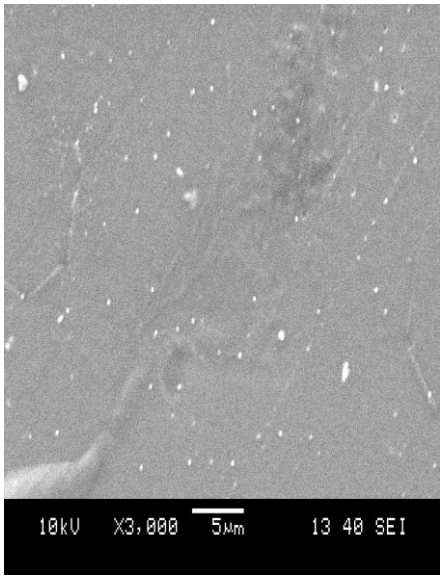


C



D

Figure 3.2 SEM images of the membranes CS/PVA/GA 1, (B) CS/PVA/GA 2, (C) CS/PVA/GA 3, (D) Ag imprinted.

3.3 Crystallinity by X-Ray Diffraction (XRD)

X-ray diffraction characterization (XRD) patterns were obtained from pure chitosan, non imprinted membrane and Ag imprinted membrane samples.

PVA diffraction patterns were analyzed based on the monoclinic unit cell (Bunn, 1948) and chitosan patterns on the orthorhombic and monoclinic unit cell (Clark & Smith, 1936; Mazeau, Winter, & Chanzy, 1994; Ogawa, Yui, & Okuyama, 2004). Chitosan presents an orthorhombic unit cell of dimensions α = 0.826 nm, b = 0.95 nm and c (fiber axis) = 1.043 nm with 2–fold helical chains stabilized by hydrogen bond with the gauche–trans orientation. There are direct hydrogen bonds between adjacent chains along the α–axis, which makes a sheet structure parallel to the ac-plane and no hydrogen bond between the sheets. Chitosan and chitosan derived networks usually exhibit a semi–crystalline structure due to free–energy balance caused by hydrogen bonding formation (Arvanitoyannis, 1999; Ogawa, Yui, & Miya, 1992; Okuyama, Noguchi, Hanafusa, Osawa, & Ogawa, 1999).

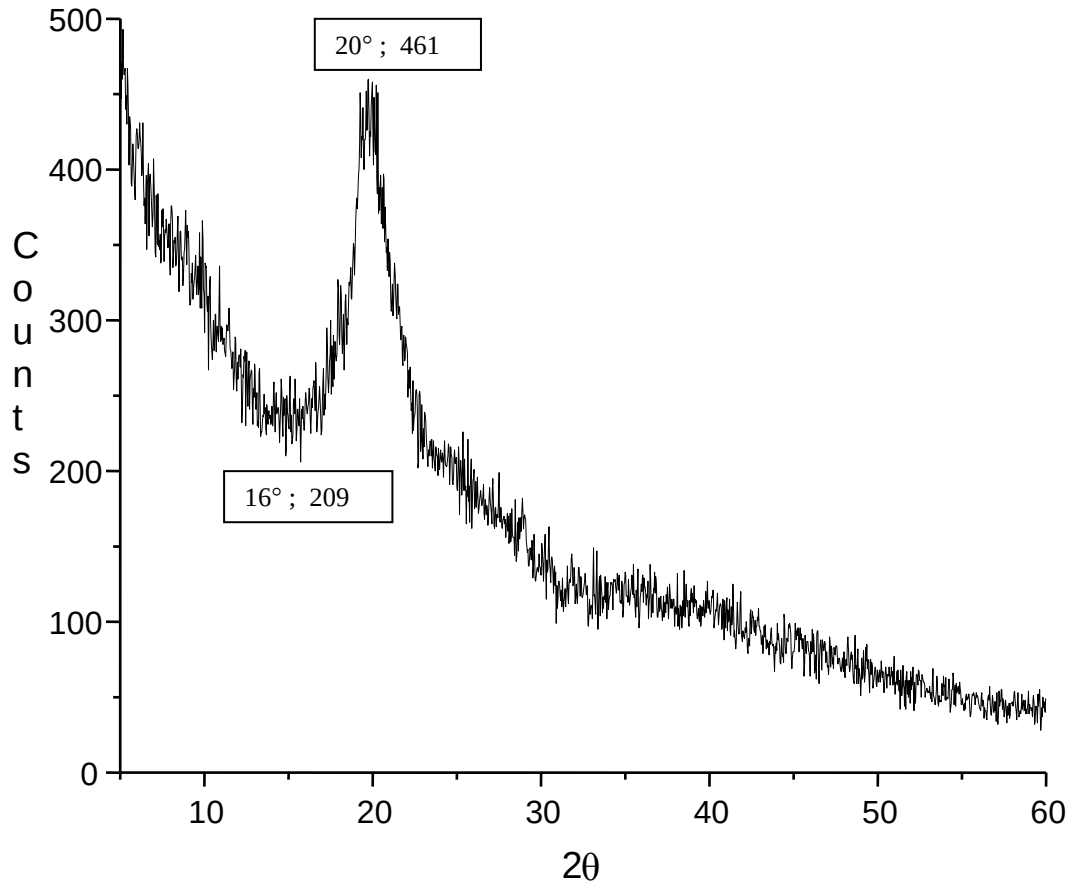


Figure 3.3 XRD pattern of chitosan

The chitosan diffraction pattern presented above. There was one major peak, characteristic of polymer crystallinity at 20° resulting from the crystalline phase and a broad region under this peak was related to the predominant amorphous phase.

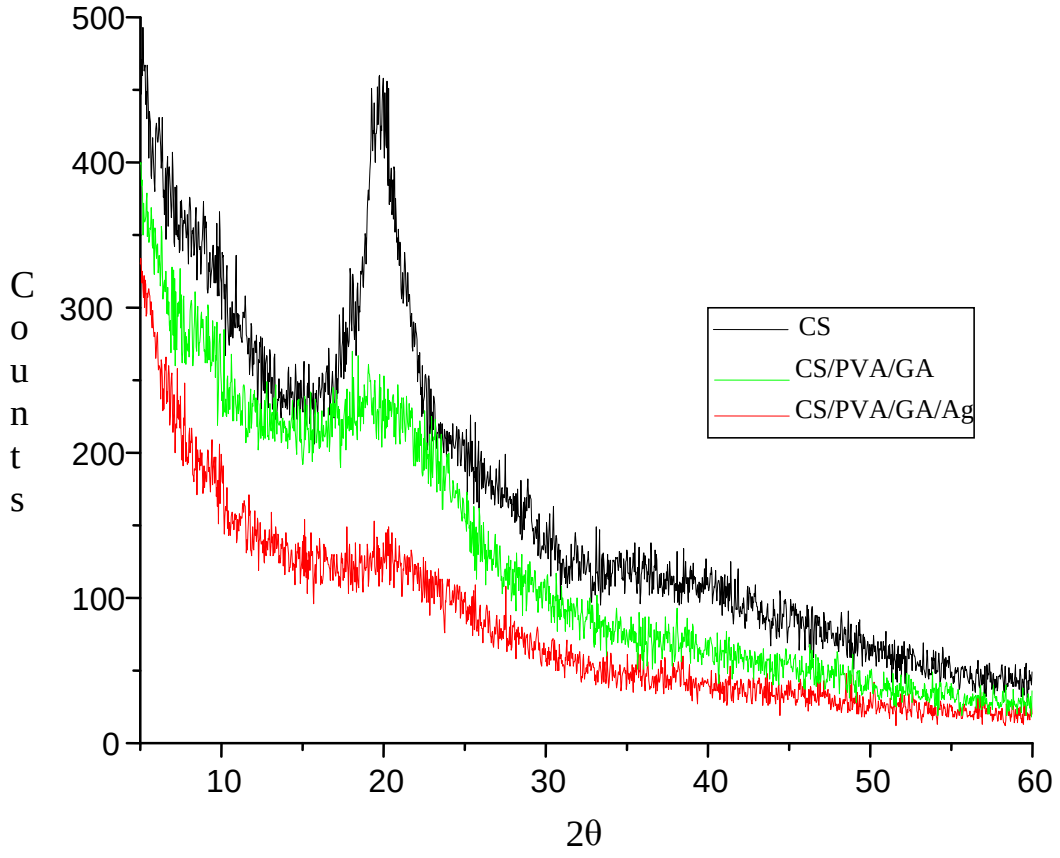


Figure 3.4 XRD patterns of chitosan (CS), CS/PVA/GA, CS/PVA/GA/Ag

Figure 3.4 shows the diffractogram of pure chitosan, non-imprinted and Ag-imprinted membranes. While chitosan had higher average crystallinity index, non-imprinted and Ag-imprinted membranes had reduced crystallinity because of GA cross linking and Ag ion imprinted. So, the cross linking inhibits close packing of the polymer chains by reducing the degree of freedom in the 3-D conformation, limiting or even preventing the formation of crystalline regions (Beppu, Vieira, Aimoli, & Santana, 2007).

3.4 Swelling Tests

Swelling experiments were conducted with CS/PVA blends with different GA concentrations in distilled water at 37°C. The results have revealed a strong influence of cross linking on the swelling volume, from about 28 % with 9.5 % GA1 and it dropped to 13% and 10%, with 13.5 % GA2 and 17.5 % GA3, respectively. The fact is attributed to a more rigid network formed by the inter-intra polymer chain reactions that have occurred, reducing the flexibility and number of hydrophilic groups of hydrogel which is unfavorable to the swelling rate. So, these results are corresponding to the hydrogel mechanism. Before GA reaction, the PVA chains are physically entangled with the chitosan chains, forming a hydrogel network. In the sequence, when the GA content was increased the chemical cross linking has occurred, forming covalent bonds among chains, fixing and reducing polymer mobility, which resulted in a lower swelling rate, which in this case was less than half of the blend without chemical cross linking.

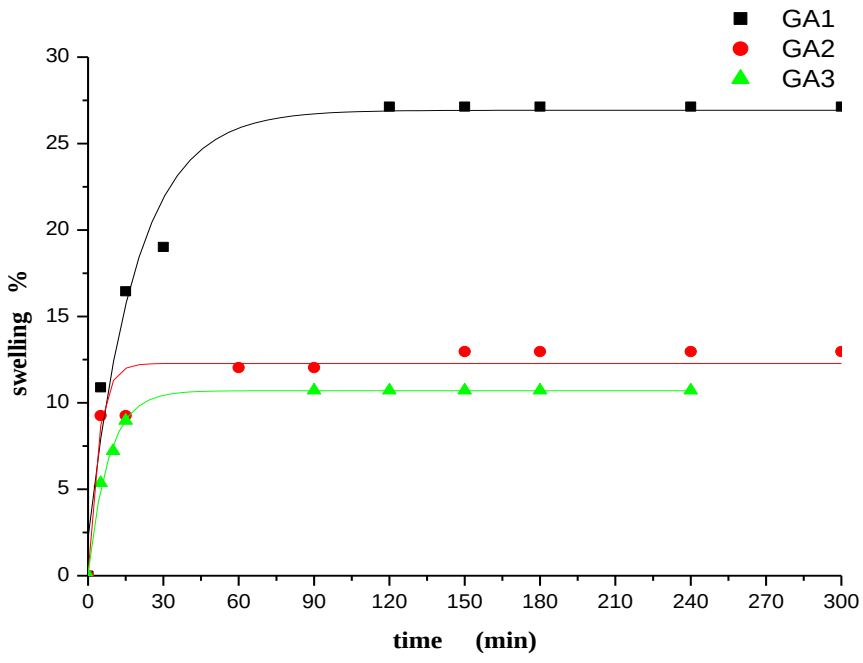


Figure 3.5 Swelling degree of CS/PVA blends with different GA cross linking content in distilled water.

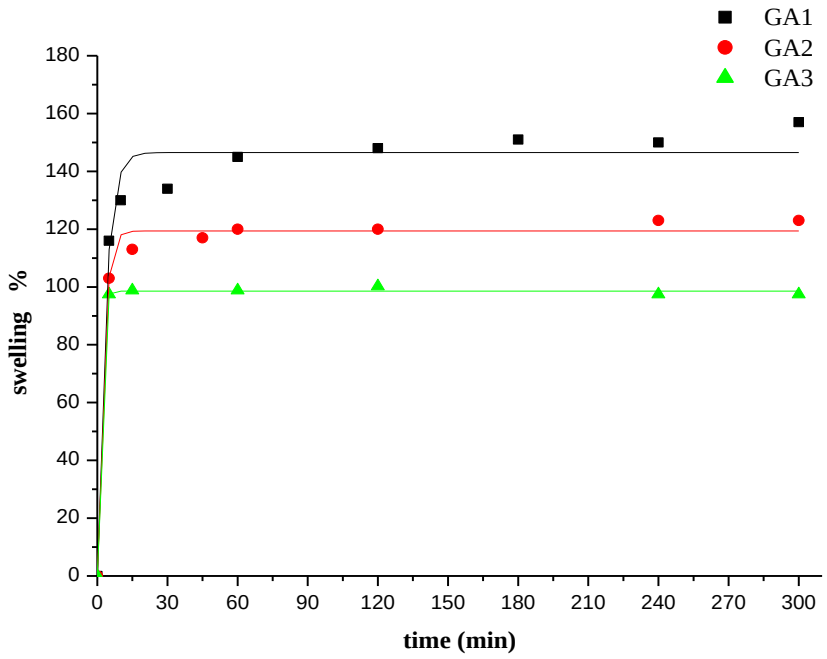


Figure 3.6 Swelling degree of CS/PVA blends with different GA cross linking content in SGF

Swelling experiments were investigated with CS/PVA blends with different GA concentrations in SGF (simulated gastric fluid) at 37°C. When GA concentration was increased, the swelling degree of membranes reduced. GA1 contend was less than GA3.and swelling degree of GA1 was higher than GA3. Also, the swelling degree in simulated gastric medium was higher than distilled water.

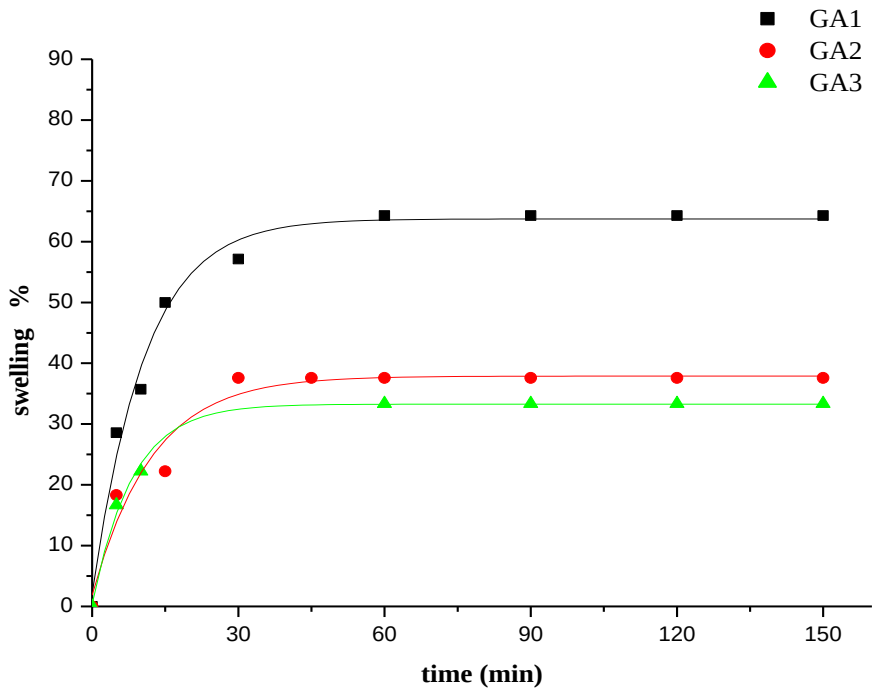


Figure 3.7 Swelling degree of CS/PVA blends with different GA cross linking content in PBS

Swelling experiments were investigated with CS/PVA blends with different GA concentrations in PBS (phosphate buffer solution) at 37°C. The results showed that swelling degree reduced when GA concentration was increased. While 17.5 % GA (GA3) had about 34% swelling degree, 9.5 % GA (GA1) had about 64 % swelling degree. Swelling degrees in PBS were more than in distilled water, but, they were less than in SGF.

3.5 Tollen Tests

Tollen test was applied to investigate unreacted glutaraldehyde in membranes. Firstly, the membranes were immersed in 50 mL of 1 M NaOH for 1 day to eliminate unreacted GA. After that, 1.5 mL of this solution was taken at certain intervals and applied to tollen test. The results show that tollen test was negative, and that means all of glutaraldehyde was cross linked with – NH_2 in chitosan or –OH in PVA.

Table 3.1 Results of Tollen test

Time for Removal Unreacted GA	Results
1 hours later	*Negative (-)*
2 hours later	*Negative (-)*
3 hours later	*Negative (-)*
4 hours later	*Negative (-)*
5 hours later	*Negative (-)*
6 hours later	*Negative (-)*
7 hours later	*Negative (-)*
8 hours later	*Negative (-)*
9 hours later	*Negative (-)*
10 hours later	*Negative (-)*
24 hours later	*Negative (-)*

3.6 Thermogravimetric Analysis (TGA)

TGA and DTG analyses were carried out in order to determine the effect of chemical modification on thermal stability of the membranes. Figure 3.8 and 3.9 shows the TGA and DTG curves of GA1, GA2 and GA3. On the TGA curves, we can see that the degradation of GA1, GA2 and GA3 are different. The first and second stage decomposition of GA1 was in the range 44-85, and 225-331°C with a mass loss of 2.8% and 47.9%, respectively. The decomposition of GA2 was in the range 34-139, 214-341, and 432-500°C with a mass loss of 6.6%, 47.3%, and 4.2% respectively for the first, second and third stages. The first and second stage decomposition of GA3 was in the range 38-118, and 216-262°C with a mass of 2.1% and 32.5%, respectively. The first stage of decomposition for GA1, GA2 and GA3, was mainly due to the loss of water that was physically adsorbed on material surface. From DTG we can calculate the temperature at which maximum mass loss occurs. The maximum decomposition temperature for the first and second stages were 67, 267°C respectively for GA1, the corresponding values for GA2 were 68, 265, 471°C and for GA3 were 65, 262°C.

According to TGA and DTG analyses, total mass loss of GA3 (32% mass loss of the dry membrane occurred at 600 °C) was less than GA1 and GA2 (53% and 61% mass loss of the dry membrane occurred at 600 °C). These results showed that GA3

was more thermally stable than GA1 and GA2. GA3 included more glutaraldehyde and therefore it could indicate that glutaraldehyde leads to the thermal stability of the membranes.

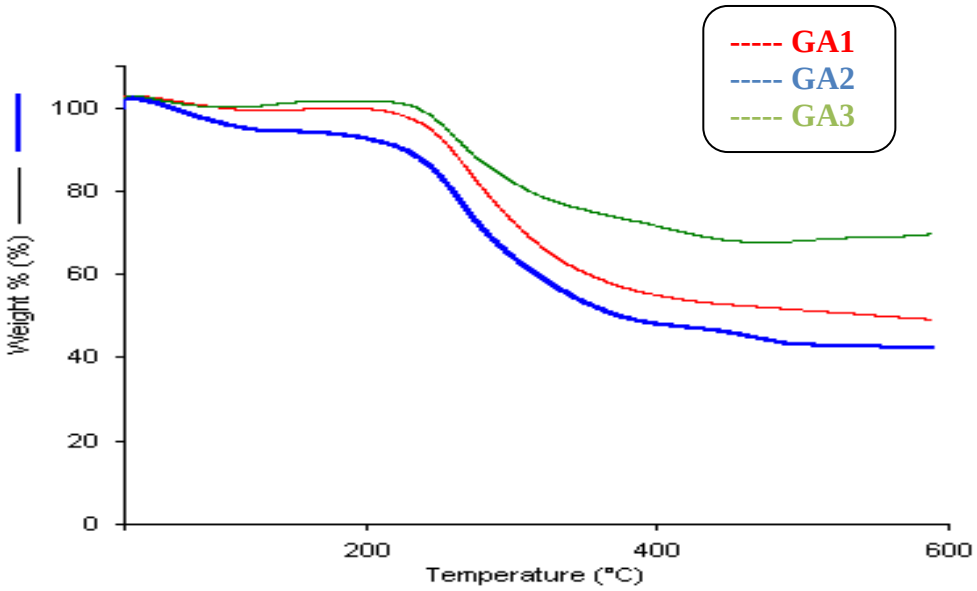


Figure 3.8 Thermogravimetric curves of GA1, GA2 and GA3.

Table 3.2 Results of thermogravimetric analysis

Sample	First Stage		Second Stage		Third Stage		Mass Remaining After 600°C
	T (°C)	Mass Loss %	T (°C)	Mass Loss %	T (°C)	Mass Loss%	
GA1	67	3	267	48	-	-	49
GA2	68	7	265	47	471	4	42
GA3	65	2.1	262	32.5	-	-	68

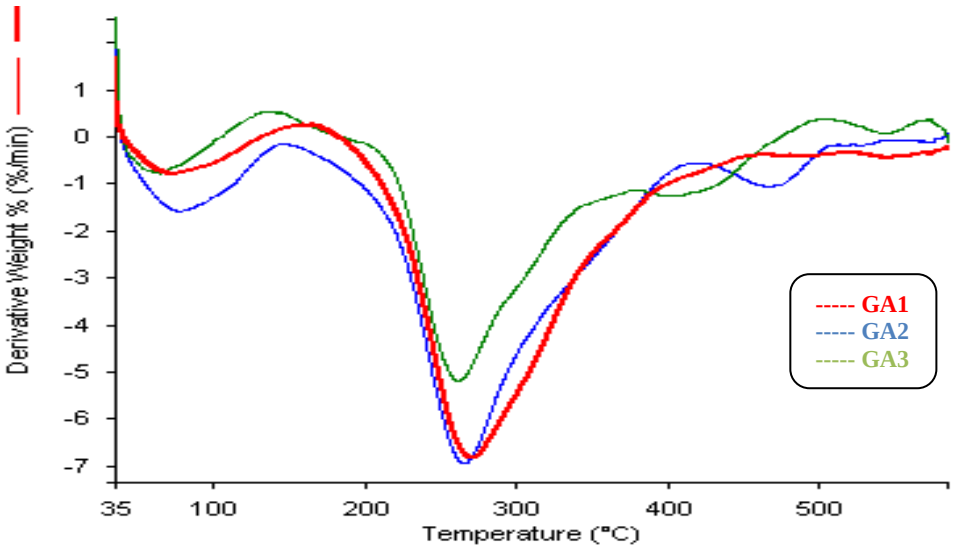


Figure 3.9 DTG curves of GA1, GA2 and GA3.

3.7 Recovery of Ag (I) Experimental Results

The amounts of Ag-ions were determined by the atomic absorption spectroscopic analysis as in section 2.3.5 under the conditions of Table 2.1.

For the FAAS measurements, calibration curve in the determination of the Ag+ ion concentration was given in Figure 3.10.

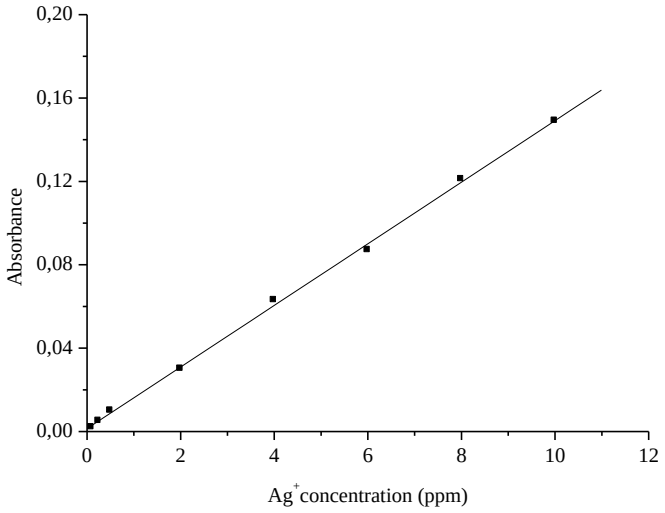


Figure 3.10 Calibration curve of Ag^{+} ion

The equation of the calibration curve was Absorbance=0.01478[Ag^+]+0.0014 with the R^2=0.9995.

The results of the recovery of Ag^+ from the samples in solution by using EDTA and thiourea was presented in Figure 3.11.

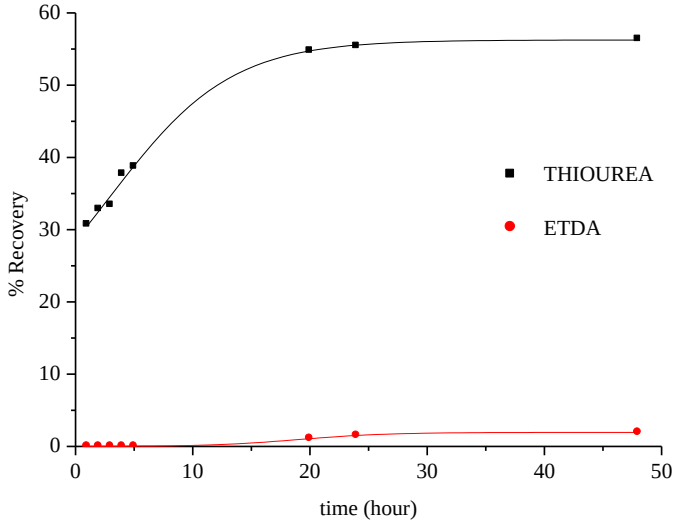


Figure 3.11 Recovery of Ag^+ ion from the samples

It can be seen from the Figure 3.11 that it was found that 0.5 M thiourea in 1M HCl solution has higher removal capacity for Ag (I) ions when compared with EDTA.

CHAPTER FOUR

CONCLUSIONS

The ion-imprinting method is a useful technique for the preparation of adsorbents for the selective separation of ions, such as silver from aqueous solutions. Ion-imprinted adsorbent is a new type of carrier that can considerably enhance the removal capacity and the selectivity of ions.

Using blends of chitosan and polyvinyl alcohol as film-forming materials, the metal ion imprinted membrane was prepared with silver ions as template (Ag (I)-IIM). Also, non-imprinted membranes for different GA content were synthesized.

FTIR spectra of the samples were shown in Figure 3.1. As can be seen from the Figure that the Schiff base formation as verified by the 1634 and 1550 cm^{-1} bands associated with the C=N and NH_2 groups. It has been also observed that the imine group was formed by the nucleophilic reaction of the amine from chitosan with the aldehyde due to the bands at 1634 cm^{-1}.

According to SEM analyses in Figure 3.2, Ag imprinted membrane was coated the surface of membrane, whereas non imprinted membranes had holes on the surface of the membranes. It was also observed some thiny and bigger white flakes or dots on the surface of membranes in films. These may be attributed to silver ions and unsoluble PVA traces. Also lower and higher glutaraldehyde concentration had different morphology because of degree of cross linking.

XRD results were shown in Figure 3.3. As can be seen from the Figure that chitosan, non imprinted and imprinted membranes had different crystallinity index. Cross linking of CS/PVA with GA was caused a decrease in the intensity of the peak at $2\theta=20°$. On the other hand, addition of Ag^+ ions on this sample was resulted severe decrease in the signal intensity. This may also indicate that the influence of silver ion and also the amount of cross linker GA would be affective on the basic reflection of CS at 20°.

Swelling properties of the samples were given in Figure 3.5-3.7. Swelling tests showed that when the amount of cross linker GA was increased, swelling was reduced because of forming covalent bonds among chains, fixing and reducing polymer mobility. In addition, swelling ratio in SGF was found higher than in distilled water and in PBS.

In order to control the cross linking procedure, Tollen test which is a quality test for aldehyde recognition was carried out. The test results showed in Table 3.1 that there was no unreacted glutaraldehyde in membranes.

In order to investigate thermal stability of membranes, TGA/DTG analyses were applied and the results were given in Table 3.2. The results exhibited that when glutaraldehyde content was increased, thermal stability of membranes were increased due to the formation of the C=N bonds in the cross linking.

The recovery of Ag (I) ions was achieved by desorption in thiourea/HCl solution. On the other hand, recovery of Ag^{+} ions with EDTA was not achieved.

As a conclusion, the CS/PVA/GA hydrogel membranes may be useful for the preconcentration of the Ag^{+} rich waste waters. It is also recommended the further experiments about the ion selectivity purposes, especially with Au and Ag mixed solutions.

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