Chapter 1

Introduction

1.1 Hydrogels

A Hydrogel is a network of polymer chains which are hydrophilic in nature but insoluble in water. It absorbs substantial amount of water due to its physical and chemical cross- linking. It is solid, jelly-like material that can have properties ranging from soft and weak to hard and tough.

Hydrogel materials are colloidal gels in which water is in dispersed medium and are able to absorb a large amount of water compared with general water absorbing substances. The absorbed water is hard to remove under pressure because water gets bounded with the water structure chemically and physically .The ability of the Hydrogels to absorb water to an extent is due to the presence of hydrophilic functional groups such as –OH, -COOH,-$CONH_2$ between network chains. Chemically the structure of Hydrogels can be assumed as all polymer chains which are cross-linked to each other by covalent bonds and thus the Hydrogels is one molecule regardless of its size. The other forces in Hydrogels matrices are hydrogen bonding, electrostatic forces and London forces. The Hydrogels are both naturally occurring and synthetic one. These are unique compounds due to their interesting properties such as high bio-compatibility and lack of toxicity. The content of water in Hydrogels affects many properties of Hydrogels significantly as their mechanical strength gets highly decreased with increase in water content in most of the cases. The Hydrogels have many similar

properties with living tissues as they are soft and rubbery and have low interfacial tension with water or biological fluids and have high water content. The pore size on the surface of Hydrogels increase with increase in water content. This property of Hydrogels permits them for the use of drug delivery. The pore size of Hydrogel is also important for separation of protein molecules from their mixture. Molecules of different size are able to diffuse into Hydrogels and out of Hydrogels with controlled rate. Factors such as polymer composition, water content, cross linking density, crystallinity are used to control the rate of release of drugs from the Hydrogels.

1.2 Composition Of Hydrogels

Gels consist of a solid three-dimensional network that spans the volume of a liquid medium and ensnares it through surface tension effects. This internal network structure may result from physical bonds (physical gels) or chemical bonds (chemical gels), as well as crystallites or other junctions that remain intact within the extending fluid. Virtually any fluid can be used as an extender including water (Hydrogels), oil and air (aero gels). Both by weight and volume, gels are mostly fluid in composition and thus exhibit densities similar to those of their constituent liquids.

Edible jelly is a common example of a Hydrogel and has approximately the density of waters of gels.

Hydrogels (also called aqua gel) is a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent (they can contain over 99% water) natural or synthetic polymers. Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content.

1.3 Classification Of Hydrogels:

Hydrogels may be of natural origin or prepared by co-polymerization of two monomers or with some natural polymers like starch, cellulose or gelatin with suitable monomer. These are classified with respect to their origin, composition and mode of synthesis.

1.3.1 Classification Based On Preparation Methods:

Homopolymer Hydrogels (one type of hydrophilic mer), Copolymer Hydrogels (two types of mers, at least one hydrophilic), Multi-polymer Hydrogels (more than three types of mers), Interpenetrating polymeric Hydrogels (swelling a network of polymer1 in mer2, making intermeshing network of polymer1 and polymer2).

1.3.2 Classification Based On Ionic Charges:

These are classified as Neutral Hydrogels, Anionic Hydrogels, Cationic Hydrogels and Ampholytic Hydrogels

1.3.3 Classification Based On Structures:

These are classified as Amorphous Hydrogels (chains randomly arranged), Semi-crystalline Hydrogels (dense regions of ordered macromolecules, i.e. crystallites) and Hydrogen bonded Hydrogels

1.3.4 Classification Based On Stimuli Responses:

These are classified as Intelligent or smart Hydrogels

1.3.5 Classification Based On Cross-Linking:

Chemically cross-linked and Physically cross-linked Hydrogels are there.

1.3.6 Classification Based On Origin:

These are classified as Homopolymer hydrogels and Co- polymer hydrogels

1.4 Chitosan Based Hydrogels

With Chitosan based Hydrogels, this classification is not entirely suitable. There is modified classification for Chitosan Hydrogels i.e. the separation of chemical and physical Hydrogels.

The chemical Hydrogels are formed by irreversible covalent links in a covalently cross linked Chitosan Hydrogels. Physical Hydrogels are formed by various reversible links. These can be ionic interactions as in the case of ionically cross-linked Hydrogels and poly-elecrolyte complexes or secondary interactions as in Chitosan/poly(vinyl alcohol)(PVA) complexed Hydrogels, grafted Chitosan Hydrogels and entangled Hydrogels.

1.5 More About The Chitin And Chitosan

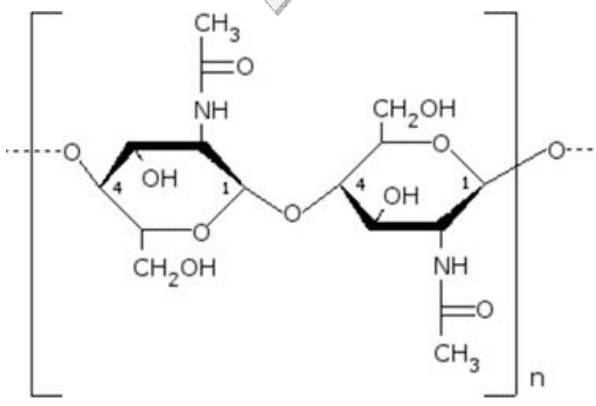
Chitin is a white, hard, inelastic, nitrogenous polysaccharide found in the exo-skeleton as well as in the internal structure of invertebrates. The waste of these natural polymers is a major source of surface pollution in coastal areas.[7.19]

Commercially chitin and Chitosan are of great importance owing to their relatively high percentage of nitrogen(6.89 per cent)compared to synthetically substituted cellulose.

De-calcification in dil. Aqueous HCl solution (3% to 5% w/v HCl at room temperature)

De-proteination in dil. NaOH aqueous solution (3% to 5% w/v NaOH, 80^0C to 90^0C for a few hrs. or at room temperature)

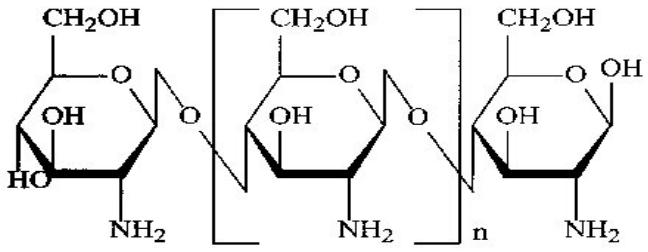
De-colorization in 0.5% w/v $KMnO_4$ aqueous and oxalic acid aqueous



Chitin

De-acetylation in hot concn. NaOH solution

(40% to 50% w/v NaOH at 90^0C to 120^0C for 4 to 5 hrs.)



Chitosan

The following four steps in chronological order of the process are needed to produce Chitosan from crustacean shells:

(1) De-proteinization

(2) De-mineralization

(3) De-colouration

(4) De-acetylation

Crustacean shells ⟹Size reduction ⟹Protein separation ⟹ Dil.(NaOH) Washing ⟹ Demineralisation ⟹ dil. (HCI)Washing and Dewatering ⟹ Decolouration ⟹ Chitin ⟹ Deacetylation(NaOH sol.)⟹ Washing and Dewatering ⟹Chitosan

1.6 Applications Of Chitosan

1.6.1 Industrial Applications Of Chitsoan

Due to its chemical and physical properties, Chitosan is used in different array of widely different products and applications. In different applications ,different properties are required. These properties change with e.g degree of de-acetylation and molecular weight as well.

1.6.2 Cosmetics

Chitin and Chitosan are fungicidal and fungistatic in nature. Chitosan is compatible with many of the biologically active components incorporated in cosmetics product composition. Composition based on Chitosan and other hydrocolloids containing anti-oxidants, anti-allergic and anti-inflammatory substances of vegetable origin. Chitosan and its derivatives have two advantages that make it good candidate for skin care. One being their positive electrical charge and the another the molecular weight of most of Chitosan products are so high that they can not penetrate the skin.

1.6.3 Water Engineering

Due to its polycationic nature, Chitosan can be used as flocculating agent. It can also act as chelating agent and heavy metal trapper. Chitosan molecules agglomerate largely anionic wastes in solution to form precipitates. Hence it acts as a flocculant for food processing waste. Chitosan can compete effectively with synthetic resins in the capture of heavy metals from processing water. Chitosan and chitin based products are effective in removal of petroleum and petroleum products from waste water. Regenerated chitin, Chitosan and chitinous membranes could be widely used for Osmosis, Reverse osmosis, De-salination, Micro-filtration and Dialysis.

1.6.4 Paper Industry

Biodegradable chitin and Chitosan can strengthen recycled paper and can increase the environmental friendliness of packaging and other products. It is already used in the manufacture of paper because it resembles with cellulose the main constituent of plant wall. It also saves chemical additives and increases output. The paper produced with Chitosan has a smoother surface and is more resistant to moisture**.**

1.6.5 Textile Industry

Derivatives of chitin are used to impart anti-static and soil repellant characteristics to the textiles. It can be used in printing and finishing preparations. Chitosan has made remarkable contribution to textile, sutures,threads and fibres.

1.6.6 Food Processing

There is wide use of Chitosan in food industry as it is non-toxic for warm blooded animals. Chitin and Chitosan act as solid support for the entrapment of the whole microbial, animal or plant cell immobilization. Chitin has been used in immobilization of the enzymes. In India, incorporation of chitin in poultry feed at level of 0.5 percent decrease the food consumption ratio and increase the body weight by 12 percent in comparison with birds fed on chitin free diet.

1.6.7 Agriculture

Chitin treated seeds have been found to have accelerated growth. Chitin additions to the potting mixture/soil resulted in significant reduction in root knot worm and suppression of fungal pathogens.

1.6.8 In Dialysis

Chitosan membranes have been proposed as an artificial kidney membrane because of their suitable permeability and high tensile strength. A series of membranes prepared from chitin and its derivatives improved dialysis properties. Chitosan is hemostatic, i.e. causes clots. The people who are at risk of internal haemorrhage can be dialysed with Chitosan membranes.

1.6.9 Tissue Engineering

Tissue engineering has been developed to construct artificial tissues that can mimic the natural ones by combining the modulated cells with different types of scaffolding materials. Chitosan and its derivatives have been studied for

use in several biomedical application including wound dressing, drug delivery system and space filling implants. Chitosan has been found to have an accelerating effect on the tissue engineering process because of its poly-cationic nature. This enhances the cell attraction to this polymer. It has been found that the degree of cell attachment also depends on the percent of de-acetylation of the Chitosan. Many efforts have been made to use Chitosan as scaffolding material.

1.6.10 Burn Treatment

It is very good for burn treatment because it forms tough water absorbing biocompatible films. These films can be formed directly on the burns by the application of an aqueous solution of Chitosan acetate. It has excellent oxygen permeability. This is important to prevent oxygen deprivation of injured tissues. Chitosan films have the ability to absorb water and are naturally degraded by body enzymes. This fact means that the Chitosan bandage need not be removed.

1.6.11 Artificial Skin

The design for artificial skin which is applicable to long term chronic use focuses on a non-antigenic membrane which performs as a bio-degradable template for the synthesis of neo-dermal tissues. It appears that the Chitosan polysaccharides having structural characteristics similar to glycosamino glycans can be considered for developing such sub stratum for skin replacement.

1.6.12 Ophthalmology

Chitosan has replaced the synthetic polymers in ophthalmological applications. It possesses all the characteristics required for an ideal contact lens. The contact lenses are made of partially de-polymerised and purified squid pen Chitosan by spin casting technology. These contact lenses are clear, tough and possess other required physical properties such as modulus, tensile strength, retention of water content and oxygen permeability.

1.6.13 Drug Delivery

Since chitin and Chitosan do not cause any biological hazard and are inexpensive, these polymers might be suitable for use in the preparations of dosage of commercial drugs. Controlled release technology emerged during 1980 as a commercially sound methodology. The achievement of predictable and reproducible release of an agent into a specific environment over an extended period of time has many significant merits. The most significant merit would be to create a desired environment with optimal response, minimum side effect and prolonged efficacy. Chitin and Chitosan controlled delivery system are at developing stage and can be used for wide variety of reagents in several environments.

1.7 Networks Of Chitosan Hydrogels

Hydrogels based upon polysaccharide structure can be divided into three classes depending upon their network:

(a) Entangled network

(b) Covalently cross-linked network
(c) Network formed by secondary interactions

The physical, chemical and biological properties of chitin and Chitosan depend mainly on two parameters:

(a) Degree of de-acetylation(DD)
(b) Molecular weight distribution

Both of which are affected by the source of Chitin and the method of preparation.The DD also plays a significant role in affecting the most of Chitosan. A lower DD leads to higher molecular weight.

Chitosan is very difficult to dissolve in water, alkaline solution or common organic solvents. This is due to the formation of intermolecular hydrogen bonds of its molecules. It is soluble to some extent in dilute aqueous acid solutions. Due to the presence of amino groups in its molecular structure it gets protonated in the aqueous acid solution and is soluble.

In the presence of solution of Chitosan, an aqueous organic acid is always used as solubilising agent. The level of solubility of Chitosan in dilute acid depends on its molecular weight and degree of de-acetylation. The viscosity is an important factor in the conventional determination of molecular weight of Chitosan and its commercial applications.

Chitosan is a natural polysaccharide comprising copolymers of glucosamine and

N-acetylglucosamine and can be obtained by the partial de-acetylation of chitin from crustacean shells, the second most abundant natural polymer after cellulose. Chitosan has been widely used in vastly diverse fields, ranging from waste management to food processing, medicine and biotechnology. It becomes an interesting material in pharmaceutical applications due to its biodegradability and biocompatibility and low toxicity.

The use of Chitosan in novel drug delivery as muco-adhesive, peptide and gene delivery as well as oral enhancer have been reported in the literature. Chitosan exhibits myriad biological actions such as hypocholesterolemic, antimicrobial and wound healing properties. Since Chitosan is a new substance it is important to carry out precise standardization for its pharmaceutical and biomedical applications like other auxiliary substances. Chitosan can be characterized in terms of its quality, intrinsic properties (purity, molecular weight, viscosity and degree of de-acetylation) and physical forms. Furthermore the quality and properties of Chitosan product may vary widely because many factors in the manufacturing process can influence the characteristics of the final product.

Chitosan is commercially available from a number of suppliers in various grades of purity, molecular weight and degree of deacetylation . It is reported that the degree of de-acetylation is one of the more important chemical characteristics which could influence the performance of Chitosan in many of its applications. In addition the degree of de-acetylation which determines the content of free amino groups in the polysaccharides can be employed to differentiate between chitin and Chitosan. For instance chitin with a degree of deacetylation of 75% or above is generally known as Chitosan. The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin leaving behind a complete

amino group (-NH_2) and Chitosan versatility depends mainly on this high degree chemical reactive amino groups.

There are methods available to increase or decrease the degree of deacetylation. For example increase either in temperature or strength of sodium hydroxide solution could enhance the removal of acetyl groups from chitin, resulting in a range of Chitosan molecules with different properties and hence its applications. Since the degree of deacetylation depended mainly on the method of purification and reaction conditions, it is therefore essential to characterize Chitosan by determining its degree of deacetylation prior to its utilization.

Various methods are used for the determination of the degree of deacetylation of Chitosan. These methods include:

(1) Ninhydrin Test

(2) Linear Potentiometric Titration

(3) Near-Infrared Spectroscopy

(4) Nuclear Magnetic Resonance Spectroscopy

(5) Hydrogen Bromide Titrimetry

(6) Infrared Spectroscopy

(7) First Derivative UV-Spectrophotometry.

Some of the methods are either too tedious, costly for routine analysis. Many limit the range of degree of de-acetylation (DD) to which they are applicable.

Lately, the first derivative UV-spectrophotometry was advocated for the degree of de-acetylation determination. The DD values of Chitosan appeared to be highly associated with the analytical methods employed.

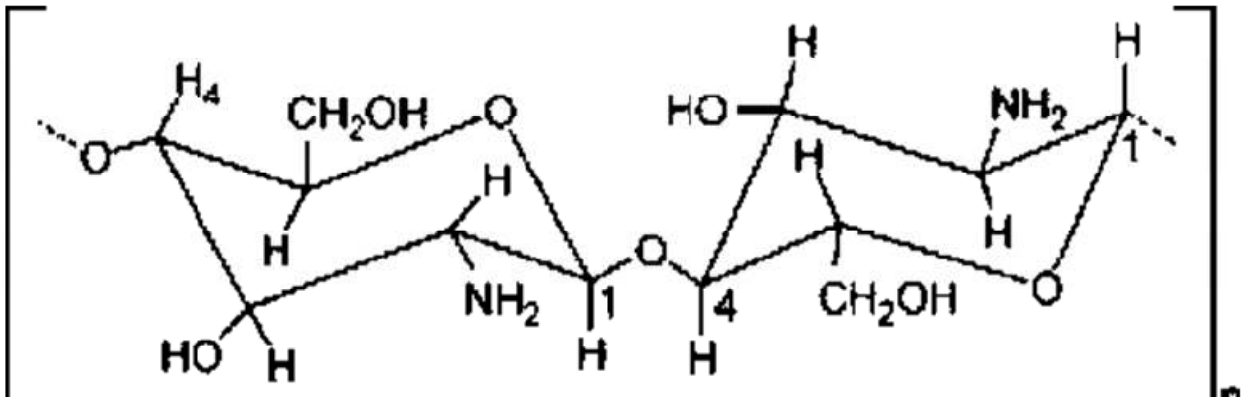
1.8 Covalently Cross-Linked Chitosan Hydrogels

Hydrogels based upon covalently cross linked Chitosan can be divided into three types with respect to their structure

(1) Hybrid polymer network (HPN)

(2) Semi-interpenetrating polymer network (SIPN)

(3) Full -interpenetrating polymer network (IPN)



Structure of Chitosan

With the discovery and synthesis of large number of new therapeutic moieties, a need for development of special vehicle for their delivery has also come into view. New systems are needed to deliver genetically engineered pharmaceuticals, *viz.* protein and peptides and to improve the therapeutic efficacy and safety of drugs administered by conventional method. In the recent years,

considerable attention has been focused on development of new drug delivery systems. The oral route of drug delivery remains the preferred and most patient-convenient means of drug administration. Oral administration presents a series of attractive advantages over other routes of drug delivery. These advantages are particularly relevant for the treatment of pediatric patient and include the avoidance of pain and discomfort associated with injection and the elimination of possible infections caused by inappropriate use or reuse of syringe.

Moreover, oral formulations are less expensive to produce, because they do not need to be manufactured under sterile conditions. But the major problem is that oral route is burdened with physiological variability which makes drugs ineffective for the treatment of various diseases. Over the past 20 years, advances in oral modified-release technologies have been largely driven by development of improved bio-compatible and bio-degradable polymeric materials for controlling release rates.

In the recent years interpenetrating polymeric networks (IPNs) [7.20]Hydrogels have generated considerable interest as a biomaterial vehicle for drug delivery. IPNs of Hydrogels encompasses the advantages of both the conventional dosage forms as well as novel drug delivery systems by offering a biocompatible, convenient and stable drug delivery system for molecules as small as non-steroidal anti-inflammatory drugs or as large as proteins and peptides.

Hydrogels are the three-dimensional network polymers that are known to swell in aqueous solutions. In the swollen state they are soft and rubbery resembling the living tissue exhibiting excellent biocompatibility. Polymeric Hydrogels are of considerable interest as biomaterials in drug delivery research.

IPNs are defined as a combination of two polymers in network form, at least one of which is synthesized and/or cross-linked in the immediate presence of the other.

1.9 Interpenetrating Polymeric Networks (IPNs)

IPNs are unique “alloys” of cross-linked polymers in which at least one network is synthesized and/or cross-linked in the presence of the other. IPNs are also known as entanglements of polymer networks that are ideally held together only by permanent topological interactions. The inter-network entanglements are permanent because of chemical cross-linking and cannot be separated.

Researchers suggested that IPNs formation enable the enhancement of performance of Hydrogels. Generally IPNs are created for the purpose of combining individual properties of two or more polymers. In some cases, entirely new properties are exhibited by the IPN that are not observed in either of the two single networks alone. The development of interpenetrating network polymers is attractive because IPNs provide free volume space for the easy encapsulation of drugs in the three-dimensional network structure which are obtained by cross-linking of two or more polymer network. Various properties of IPNs such as porosity, bio-adhesiveness, elasticity, swelling and stimuli-responsive behavior can be controlled by the appropriate choice of the network-forming polymers and suitable cross-linking agent and its proportion.

IPNs can be prepared by using various matrices such as poly(urethane), poly(butadiene), poly(methacrylic acid), L-lysine, glutamic acid, poly (vinyl alcohol), carboxymethyl cellulose, poly(acrylic acid), gelatin, poly (vinyl pyrrolidone), alginate, dextran, xanthane, guar gum, Chitosan, poly(ethylene glycol) etc. for various applications.

These biocompatible, nontoxic and biodegradable polymers are now acquiring unique place for biomedical application for various purposes such as cartilage scaffolds, biological tissue graft, tissue engineering, wound dressing and for drug delivery.

1.9.1 Types Of IPNs

There are primarily two classification schemes used to describe IPNs viz. by synthesis and by structure.

1.9.1.1 Based On Synthesis

Simultaneous Interpenetrating Networks (SINs):

Two different combinations of monomers and/or pre-polymer plus the cross-linkers and initiators are mixed, followed by simultaneous polymerization via non-interfering reactions (Figure 1A). Both combinations of monomer and/or pre-polymers form two different intercalated networks to make the IPN. Interference is minimized if one of the polymerization process involves chain polymerization while the other involves step polymerization kinetics.

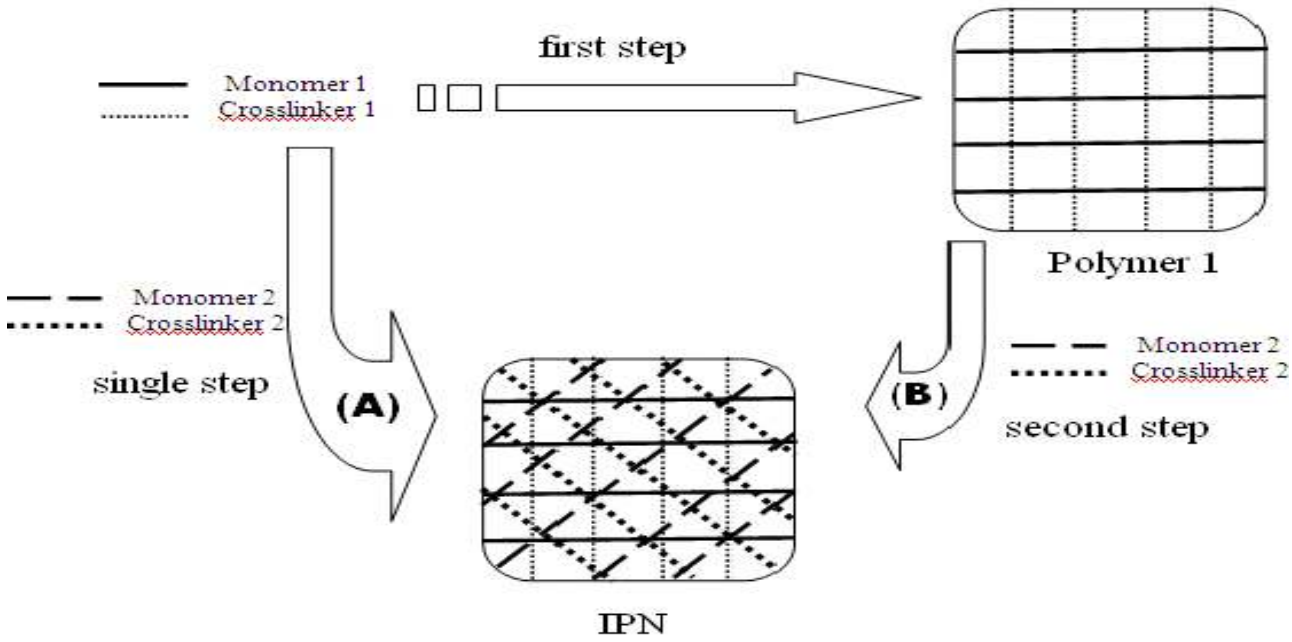


Figure1. Schematic diagram for the synthesis of: (A) Simultaneous IPN and (B) Sequential IPN

Sequential Interpenetrating Networks

In this class IPN is synthesized from mixture of different combinations of monomer and/or pre-polymer in sequential mode. Initially monomer 1 is polymerized with cross-linker 1 to produce a network. Subsequently, monomer 2 along with cross-linker 2 is swollen in and then polymerized in a sequential mode to make the IPN (Figure 1B).

1.9.1.2 Based On Structure

Full IPNs

They comprise of two or more networks that are ideally juxtaposed, which generate many entanglements and interactions between the networks (Figure2a)

Homo-IPNs

These IPNs are a special type of full-IPN, where the two polymers used in the networks are same or identical.

Semi IPNs

In these IPNs only one component of the assembly is cross linked to form network and other component is present in a linear (non-cross-linked) form (Figure 2b).

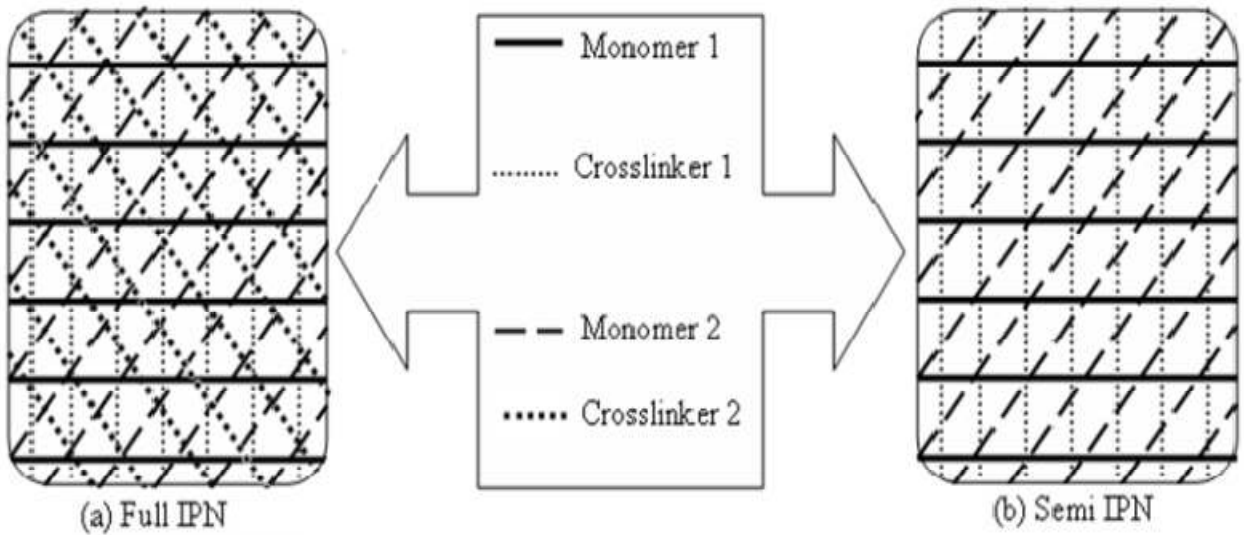


Figure 2. Schematic representation of: (a) Full IPN (b) Semi -IPN

Latex IPNs

These IPNs are prepared in the form of latex which have a core and shell structure. The polymers are synthesized by emulsion polymerization of monomer 2 together with the cross-linker and activator in the original seed latex of cross-linked polymer 1. The morphology of the IPNs depends upon how the IPNs components are polymerized.

1.9.2 Gradient IPNs*:*

In this case a film can be made with polymer network 1 predominantly on one surface and polymer network 2 on the other surface with a composition gradient existing throughout the interior.

1.9.3 Thermoplastic IPNs*:*

These materials contain physical cross-links rather than chemical cross-links (like ionic and hydrogen bond). As such they are hybrids between polymer blends and IPNs. Such cross-links may utilize block copolymers, ionomers, and/or semi-crystalline polymers. Being thermoplastic, they flow at elevated temperatures.

1.10 Characteristic Features of IPNs:

-Forms non-separable network.
- Shows adhesive property.
- Has high tensile strength.
- Forms insoluble network.
- Biocompatible IPNs formed with biocompatible polymers.
- IPNs are distinguishable from blends, block copolymers, and graft copolymers in two ways: Firstly an IPN swells but does not dissolve in solvents and secondly creep and flow is suppressed.

1.11 Structure and Properties of Poly(Vinyl Alcohol)(PVA)

Poly(vinyl alcohol) is a polymer of great interest because of its many desirable characteristics specifically for various pharmaceutical and biomedical applications. The crystalline nature of PVA has been of specific interest particularly for the physically cross-linked Hydrogels prepared by the repeated cycles of freezing and thawing.[7.6]

Poly(vinyl alcohol) has a relatively simple chemical structure with a pendant hydroxyl group. The monomer vinyl alcohol does not exist in a stable form rearranging to its tautomer acetaldehyde. Therefore PVA is produced by the polymerization of vinyl acetate to poly(vinyl acetate) (PVA_C) followed by hydrolysis of PVA_C to PVA. The hydrolysis reaction does not go to completion resulting in polymers with certain degree of hydrolysis that depends on the extent of reaction. So PVA is always a copolymer of PVA and PVA_C. The degree of hydrolysis or the content of acetate groups in the polymer has an overall effect on its properties such as solubility and the crystallizability of PVA.

The degree of hydrolysis and polymerization affect the solubility of PVA grade. PVA with high degree of hydrolysis have low solubility in water. Residual hydrophobic acetate groups weaken the intra and inter molecular hydrogen bonding of adjoining hydroxyl groups. The temperature must be raised well above 70^0 C for dissolution to occur. The presence of acetate groups also affects the ability of PVA to crystallize upon heat treatment. PVA containing high degree of hydrolysis are more difficult to crystallize.

1.12 Properties of Poly(Vinyl Alcohol)(PVA) Hydrogels

PVA must be cross-linked in order to be useful for a wide variety of applications specifically in the areas of medicines and pharmaceuticals sciences. It can be cross-linked through the use of di-functional cross linking agents. Some of the cross-linking agents that has been used for PVA Hydrogels preparation include glutaraldehyde, acetaldehyde, formaldehyde and other monoaldehydes. When these agents are used in the presence of sulfuric acid, acetic acid or methanol, acetal bridges form between the pendant hydroxyl groups of the PVA chains. For pharmaceutical applications especially when PVA is used as carrier in drug delivery, the toxic agent could alter the biological or degrade the

biologically active agent being released. There are also other toxic residual components associated with chemical cross-linking such as initiators, transfer agents and stabilizers.

The mechanical strength of cross-linked partially crystalline PVA Hydrogels has been examined and it has been observed that the mechanical strength is very low. This can be improved by increasing the crystallites along with crystallites.

1.13 Physical Cross-Linking Due To Crystallite Formation

The PVA Hydrogels have more mechanical strength when resulting material contains crystallites in addition to the cross-links. This phenomenon was examined by Peppas and Merrill[7.23] when PVA is partially crystallized by a process of dehydration and annealing. Resulting material contains crystallites in addition to cross-links. The crystalline region served as additional cross-links to redistribute external stresses.

The mechanism of Hydrogels formation involves 'physical cross-links' due to crystallites formation. This method addresses toxicity issues because it does not require the presence of cross-linking agent. Such physically cross-linked materials also exhibit higher mechanical strength than PVA gels cross-linked by chemical or irradiative techniques because the mechanical load can be distributed along the crystallites of the three-dimensional structure.

Aqueous PVA solutions have the unusual characteristics of crystallite formation upon repeated freezing and thawing cycles. The number and the stability of these crystallites are increased as the number of these freezing/thawing cycles is increased. Some characteristics of 'physically' cross-linked PVA gels include a high degree of swelling in water, a rubbery and elastic nature and high mechanical strength. In addition the properties of the gel also depends on the

(a) Molecular weight of the polymer
(b) The concentration of the aqueous PVA solution
(c) The temperature and the time of freezing and thawing
(d) The number of freezing and thawing cycles

1.14 Crystallization Of Poly(Vinyl Alcohol)(PVA) Hydrogels

On a molecular level, the crystallites of PVA can be described as a layered structure. A double layer of molecules is held together by hydroxyl bonds while weak Vander Waals forces obtain between the double layers. A folded chain structure of PVA chains leads to small ordered regions (crystallites) scattered in an unordered amorphous polymer matrix. The crystalline melting range of PVA is between 220 and 240^0C.

Usually there is minimum chain length necessary to crystallize PVA. Mandelkern et al. [7.33] reported that as the molecular weight of the polymer increased, the size of crystallites also increased. However, the character and appearance of the crystallites did not change over a molecular weight range. In particular, isotactic PVA results in less crystalline samples than syndiotactic PVA due to increased intra-molecular hydrogen bonding and thus reduced inter-molecular forces. It has been reported that atactic PVA is the most crystallizable

form with syndiotactic less crystallisable, with the isotactic form showing poor crystallinity.

Different methods have been investigated for the crystallization of PVA. Most of these methods involve heat treatment to introduce crystallites. The extent of crystallinity as well as the size of crystallites depends on the drying conditions.

1.15 Hydrogels By Freezing And Thawing Of Aqueous Poly(Vinyl Alcohol)(PVA) Solutions

To avoid cross-linking process which leads to the release of toxic agents, a physical method of gelation and solidification of PVA has been developed. This freezing and thawing process of PVA has been summarized recently.[7.6]

The preparation of pure PVA Hydrogels using freezing and thawing techniques was first reported by Peppas et al. [7.34] in 1975.The aqueous solution of between 2.5 and 15 wt % PVA were frozen at -20^0C and thawed back to room temperature resulting in the formation of crystallites. The formation of the crystallites was found to be related to the concentration of PVA in solution, the freezing time and the thawing time. Overall the crystallinity was found to increase with increasing freezing time. During the thawing process, the size of the crystallites first increased and then decreased. This is due to the breakdown of the crystalline structure. The degree of crystallinity was found to increase with increasing PVA solution concentration.

The freeze drying cycle consist of cooling a PVA solution to below -3^{0}C followed by the vacuum evaporation of water. The increase in the rate of evaporation of water results in an increase in the tensile strength. This is attributed to the strengthening of the gel by decreasing the number of imperfections which may not have been attached to the network structure.

The gels prepared by repeated freeze and thaw cycle shows unique characteristics. Using X-Ray diffraction, Scanning Electron Microscopy (SEM) ,Light Optical Microscopy and tension experiments, the structure was described as one consisting of three phases : A water phase of low PVA concentrations, an amorphous phase and a crystalline phase that restricts some of the motion of the amorphous PVA chains. The analysis of DSC endotherm peaks and X-Ray diffraction shows that the presence of bulky acetate groups can inhibit the formation of a gel. A little difference in the degree of hydrolysis can significantly impact the thermal behavior of the gels.

In preparation of PVA Hydrogels by the freezing and thawing technique, the addition of solvents has also been investigated. Hyon and Ikada et al.[7.25] prepared porous and transparent hydrated gels from a PVA solution in a mixed solvent of water and water miscible organic solvents. The concentration of PVA in solution was between 2 and 50 wt %. The organic solvent can be methyl sulfoxide, glycerine, ethylene glycol, propylene glycol and ethyl alcohol.

The method consists of cooling the solution below 0^{0}C for the crystallization of PVA followed by the subsequent exchange of the organic solvents in the gel with water. This process resulted in the formation of hydrated gels of PVA with high tensile strength, high water content and high light

transmittance. The high light transmittance was an important property of the material especially when considering the material for the contact lens applications.

As the temperature of the homogeneous solution was lowered, there was restriction in the motion of the molecules. The intermolecular interactions of PVA due to hydrogen bonding was promoted to yield small crystalline nuclei. Crystallization can proceed further as the solution remains at low temperature for longer period of time. The crystallites served as cross-links to hold the three dimensional structure together. The addition of the organic solvents served to prevent the PVA solution from freezing even below 0^0C. This allowed for PVA crystallization to proceed without significant volume expansion. Therefore the gel contains pores less than 3 μm resulting in transparent gel.

The gels prepared by freeze and thaw cycle are called Hydrogels. Upon exposing the aqueous PVA to one cycle of freezing and thawing, no destruction or covalent cross-linking of macromolecules of PVA was found. The filler can be added to grow microbial cells.

Many properties of these gels are closely related. There is a good correlation between the tack and the visco-elasticity which was useful for predicting the properties of the material for pressure sensitive adhesives in drug delivery systems. Additionally, the amount of unincorporated PVA, the density of the PVA Hydrogels, visco-elasticity and swelling behavior are also closely related .The rate of swelling of the Hydrogels is found to increase linearly with the square of the root of time immersed in water. In addition the water up take upon swelling in water increased with increasing temperature.

Gels that were prepared by freezing below 0^0C were transparent and exhibited high elasticity. Higher gelation rates were observed when compared with aqueous solutions of only PVA in water. Specifically the properties were dependent upon the ratio of dimethyl sulfoxide to water. It was also observed that gelation from the mixture occurred without phase separation below -20^0C. H-owever above this temperature phase separation plays an important role for the gelation process.

The thermal properties of these gels were classified by Hatakeyama et al. [7.26]. The water in the gels is classified as non-freezing, restrained and free water. In addition the work showed that the rate of freezing affected the size of ice crystals formed and therefore the cross-links formed. The water in the Hydrogels is free, intermediate or bound water. PVA gels prepared by low temperature crystallization contained less bound water with the concentration increasing as degree of polymerization of PVA is increased. This is attributed to the fact that the concentration of the free PVA chains which does not participate in the crystallite formation process increased with the increasing degree of polymerization. The low temperature crystallization resulted in larger free space between crystallites and larger size of crystallites than annealing.

Takamura and collaborators[7.27] examined the use of PVA gels prepared by freezing and thawing processes for applications in drug delivery. For this strong preparation of freezed/thawed PVA gel is required. It is necessary to have PVA with degree of polymerization over 1000 and a concentration of over 6% to obtain adequate strength. The strength was found to increase with the first

three cycles of freezing and thawing and then level off. The release of both hydrophobic and hydrophyllic was found to be of zero order.

The parameters which affect the characteristics of PVA gel are such as concentration of aqueous PVA, the number of freezing and thawing cycles, the time of freezing and the thawing time. Specifically the gels have been characterized in terms of swelling behavior, degree of crystallinity, the transport and release of drugs and proteins, mechanical strength and adhesive and muco-adhesive characteristics.

If high PVA molecular weight and highest number of freeze/thaw cycles are used then densest gel structure is formed. It can be prepared by freezing aqueous solution of 15wt % PVA at -20^0C for 18 hours and thawing at room temperature for 6 hours for three, four and five cycles. These gels were also found to be the strongest and most rigid. There is an initial decrease in degree of crystallinity due to melting out of smaller crystallites. This stage was followed by an increase in the crystallinity at long times. It is due to additional crystallite formation due to aging.

Hickey and Peppas et. al[7.28]examined the diffusive characteristics of PVA Hydrogels prepared by freezing/thawing techniques. Semi- crystalline PVA membrane was prepared by freezing and thawing aqueous solutions of PVA for up to ten cycles. It was observed that the PVA crystalline fraction is a function of the number of cycles and the duration of each cycle. The volume based crystalline fraction of the PVA on a wet basis ranged from 0.052 to 0.116. The equilibrium volume swelling ratio also varied from 4.48 to 9.58 with decreasing degree of crystallinity.

PVA Hydrogels can be prepared by exposing 15 to 20 wt% aqueous solution of PVA to repeated cycles of freezing for 6 to 12 hours at -20^{0}C and thawing for 2 hours at 25^{0}C.The average degree of crystallinity for the 20 wt% solution was 19.3% on a dry basis. The studies show that the work of fracture or detachment decreased with an increasing number of freezing/thawing cycles due to the increase in PVA degree of crystallinity. Maximum adhesion was increased in the two cycles. Although increasing the number of freezing and thawing cycles likely contributed to the stability of the network, the adhesive characteristics decreased.

Drug release is affected by the number of freezing and thawing cycles. The results indicated that the mucoadhesive characteristics and drug release could be optimized for a certain mucoadhesive controlled release application by controlling freezing and thawing conditions.

1.16 Biomedical And Pharmaceutical Applications Of Poly(Vinyl Alcohol)(PVA)

It can be used for numerous applications of biomedical and pharmaceutical applications. It has certain advantages which make it excellent for use in biomedical applications such as non-toxic, non-carcinogenic and bio adhesive characteristics as well as their associated ease of processing[7.16]. PVA has uncomplicated chemical structure and modifications are possible by simple chemical reactions. PVA gels exhibit a high degree of swelling in water or

biological fluids and a rubbery and elastic nature. Because of this PVA is capable of simulating natural tissue and can be readily accepted into the body. PVA gels have been used for contact lenses, the lining for artificial hearts and drug delivery applications. In addition they have been shown to have potential applications for soft tissue replacements, articular cartilage, artificial skin, artificial pancreas and hemo-dialysis membranes. PVA gels have been examined for their use in applications where blood compatibility is a major issue.

Based on such observation, PVA Hydrogels have been recommended for the re-construction of vocal cords. Cross-linked PVA gels have been considered as candidates for bio-membranes for artificial kidney applications.

Tmura et al. [7.29] examined the use of PVA gels freezing/thawing cycle as material for medical use. The gel has a water content of 80 to 90 % by weight and high mechanical strength and rubber like elasticity. The characteristics of the gel do not change after long term implantation.

The biocompatibility as well as the mechanical properties of the PVA gels in relation to their usefulness makes it suitable for the artificial articular cartilage. Its applications as lubrication, load bearing, biocompatibility and attachment of the material to the bones renders it biocompatible. It is also wear resistant.

It is also used for the use in eye contact lens materials. The transport of oxygen through cross-linked and pure PVA material is related to the overall structure of the PVA polymer. Such a technique is allowed for the preparation of the transparent gel with high mechanical strength and high water

content. This material can be used for the preparation of soft contact lens material .The transparent PVA gel with high tensile strength can be prepared by the PVA solution of 20 wt% concentration after freeze dried at -4^0C. The dried PVA gels are then swollen in an ethanol and water solution followed by the exchange of water. The resulting transparent gels had high tensile strength and elongation.

Hirai and collaborators[7.30] have also examined applications of PVA gels as shape memory properties. The contraction and the relaxation of chemically cross-linked PVA in respect of the solvents, DMSO and water shows desired properties. PVA gels prepared by freeze/thaw cycle also show shape – restoring properties. The chemical cross-links were introduced in freeze/thawed gels to enhance the shape memory characteristics. It has been explained that the added chemical cross-links served to remember the distribution of physical cross-links within the gel to restore the shape upon elongation.

PVA gels have also been proposed for the waste water treatment .The material is found to have a treating capacity for synthetic sewage of two to three times that of standard activated sludge method. When compared with poly(ethylene glycol)(PEG) and Poly(acrylamide) (PAAm) gels networks, the PVA gels have the presence of voids of several microns which were formed upon freezing. Therefore PVA gels have many more desirable characteristics such as large free volume of water and good oxygen permeability. This creates a favorable environment for the growth of micro-organisms.

1.17 Poly(Vinyl Alcohol)-Chitosan Hydrogel By Freeze Thaw Cycle.

PVA and Chitosan are synthetic and natural polymers respectively and are able to form physically cross-linked Hydrogels by a variety of techniques such as chemical cross-linking or the freeze-thaw method. A icegenic approach of blending Chitosan with PVA at different ratios followed by successive freezing-thawing operations is used. The icegenic method is performed over conventional chemical cross-linking as in the former process no chemical toxicity remains with the end product.[7.6]

In chemical cross-linking, glutaraldehye has been broadly used as an active chemical cross-linker of PVA and Chitosan due to its ability to form the formation of intra-inter chain covalent bonding. Nevertheless, this synthetic cross-linker has been reported as highly cytotoxic , which may impair the bio-compatibility of the cross-linked biomaterials.

These disadvantages can, however, be minimized by ensuring that all aldehyde functional groups have actually cross-linked with the polymeric network or they can be effectively blocked with molecules such as amino acids and proteins which are widely present in living organism serum.

On the other hand the physical cross-linking phenomenon (freeze-thaw method) is based on the existence of regular pendant hydroxyl groups on PVA that are able to form crystallites by strong inter-chain hydrogen bonding.

This method produces stable gels that are cross-linked by the presence of crystalline regions.

Advantages of physically cross-linked Hydrogels are their non-toxicity, non-carcinogenicity, high elasticity and the good biocompatibility of the resulting polymer. Chitosan is a carbohydrate biopolymer derived from de-acetylation of chitin, the main component of crustacean (e.g., shrimp, crab, lobster) exoskeleton. It is a medically important biopolymer due to its biological properties, such as its antimicrobial, hemostatic and anti-tumor activities. Its acceleration of the wound healing process, use in tissue engineering scaffolds and promise for drug delivery. It is also biodegradable, hydrophilic and biocompatible with low toxicity to mammalian cells.

Chitosan has received great attention for medical and pharmaceutical applications due to its beneficial intrinsic properties. It is one of the natural polymers that has shown a high potential in wound healing applications. It is well known for being able to accelerate the healing of wounds in humans and it has also been documented that Chitosan confers considerable antibacterial activity against a broad spectrum of bacteria.

On the other hand the PVA is a unique material even in atatic form and is semi-crystalline despite its lack of stereo regularity. In aqueous solutions with a polymer concentration of more than 1%, entangled aggregates of hydrogen-bonded PVA molecules are formed. This is a consequence of the formation of crystalline regions. It is often used in biomedical applications. It is a water-soluble synthetic polymer with excellent film-forming, emulsifying and adhesive

properties. PVA Hydrogels prepared with freeze-thaw techniques have great potential for biomedical applications. This is due to a high swelling capability often coupled with relatively good mechanical properties and biocompatibility.

Chapter 2

Literature Review

-Poly(N-isopropylacrylamide) is a temperature responsive polymer that was first synthesized in the 1950.

-The US department of commerce reported in 1973 that there is millions of tons chitin produced in US and Chitosan is of great importance because of high percentage of nitrogen compared to synthetically synthesized cellulose.

-By 1989 Bentech labs patented Chitosan salt solution applied to crops for improved freeze protection.

Recentlr Baba et al. have synthesized methylthiocarbamoyl and phenylthiocarbamoyl Chitosan derivative for selective metal ion absorption in 1992.

-Tsusume et al. have reported that the Chitosan-gadopentatic acid complex can be used for neutran capture therapy in 1984.

-Chitosan can be used as flocculating agent and it can also act as chelating agent and heavy metal s trapper. Weltroswki et al. used Chitosan N-benzyl sulphonate derivatives as sorbents for removal of Chitosan ions.

-In 1999 ,Bhavani and Dutta reported the removal of colour from the dye house effluents using Chitosan as an absorbent.

-Rhee et al. have used chitin and Chitosan as sorbent material to solid phase extraction of phenol and chlorophenol.

-In 2000 Prasitslip et al. showed how degree of de-acetylation affected in vitro cellular responses to Chitosan from two different sources of shrimp and cuttle fish.

In 2001,Jarry et al. demonstrated that Chitosan can be easily processed into porous scaffolds, films and beads.

-In 2003 Wang et al. developed a novel method to prepare polyglycolic acid – Chitosan hybrid matrices using solvents of low toxicity(DMSO and acetic acid)

-In the year 1990 Qureshi et al. prepared and characterized membranes of Chitosan and modified Chitosan

-Zhang and Zhang synthesized and characterized Chitosan/calcium phosphate composite for tissue engineering in the year 2000.

-Peppas et al. reported that Due to ionic interactions the Chitosan structure is rendered insoluble in water

- Khare and Peppas in 1995, reported the swelling of the polymers determines the drug release of the polymers.

-In 2007 clinical trials performed with Chitosan on weight loss, body weight related to cholesterol were done.

-Szepes et al. studied in 2008 the structure of the bio-materials with SEM to ensure that the structure of the Hydrogels retain their granular structure.

-Yu and Xiao in 2008 reported polymers retain their crystalline structure or they get deformed during the processing pressurization process.

-Yin et al.2008 and Kim et al., of 1992 showed the swellaebility of Hydrogels in aqueous medium or medium of specific pH .

-Schuetz et al., 2008 evaluated Hydrogels for viscosity under constant temperature of 4^0C by using cone plate type of viscometer .

-Kawaguchi 2000 reported that the Chitosan is potential candidate for the wound dressing and the controlled release of drugs.

-Chitosan is receiving interest due to its non toxicities on rats using property, odorless , biocompatible in animal tissues and biodegradable properties(Borzacchelo et al. 2001)

-Chitosan Hydrogels like other Hydrogels contain much water. Part of this water is tightly packed to the polymer (De Angelis et al. 2001)

-Shin et al. reported in 2002 that the blending of chtosan with polymers and cross-linking are both convenient and effective methods of improving the mechanical and physical properties.

-Jameela et al. carried immunization studies carried out on rats using glutaraldehyde cross linked microspheres in the year 2002.

-Studies in Helsinki have shown that individuals taking Chitosan lost an average 8% of their weight in a four week period in the year 2004.

- The linear PVA chains are cross linked using glyoxal , glutaraldehyde or borate and semi crystalline gels were prepared by exposing aqueous solutions of PVA to repeating freeze and thaw cycle by peppas and Hassan in 2000.

-Ficek and Peppas (1993) used PVA gels for the release of bovine serum albumin using novel PVA micro-particles.

-Ojawa et al. studied 3-D structure of Chitosan in the year 2004.

EXPERIMENTAL

Chapter 3

Preparation Of Hydrogels

The chemical composition of the Hydrogel matrix has a great impact on the final performance of the material. In the present study, two polymers, namely, PVA and Chitosan, were used. The former is a synthetic and hydrophilic polymer, whereas the latter is cationic and hydrophilic in nature. The internal structure of a polymer matrix greatly depends upon the number of cross-linkages present in the network. Since the cross-linkages are achieved through the cycle of freeze/thaw, so the number of cycles will affect the properties of the gel.

The chemical cross-linking leads to release of toxic agents. The method of physical cross-linking has been used. The preparation of the hydrogel by the freeze thaw cycle was reported first in the year 1975 by Peppas[7.32]. The formation of crystallites in the sample was found to be related to conc. of PVA and the freezing and thawing time.

PVA-Chitosan Hydrogels

It is based on co-polymer of Chitosan and Poly(Vinyl Alcohol)

Monomer:

Chitosan (extra pure) from SRL with degree of de-acetylation 85 and density 0.4g/cc

Poly(vinyl alcohol) from Fisher scientific having density 1.25 g/c.c

Solvent

Acetic acid glacial

CH_3COOH M W-60.05

Blulux Laboratories

Apparatus

Test tube, glass rod, Cylinder, beaker, Petri-dish, Electrical Digital balance, Oven, Magnetic stirrer ,Refrigerator

Procedure

Chitosan weighing 0.1g is dissolved in 10 ml of 1% acetic acid with glass rod and is left for 2-3 hours. 6g of PVA is mixed with 40 ml of distilled water (15% solution) with the help of magnetic stirrer and heated at 90^0C for two hours. The same is allowed to rest for half an hour so that air bubbles are removed. Mix PVA and Chitosan solution in a beaker with magnetic stirrer for an hour with heating at 50^0C and the content of Chitosan is varied with respect to Poly(vinyl alcohol).The solution of chitosan with 10-50 vol% concentration are prepared. A white transparent film will appear at the top of the solution surface .This film is to be removed and then put aluminium foil in a Petri- dish and pour the solution into it.

Now freeze this solution at -6^0C overnight. Then thaw the same in an oven for 6 hours at 30^0C and the same cycle is repeated 3 times. This is termed as Freeze thaw cycle and a white transparent jelly like mass is formed in the Petri-dish. Now dip into one molar dil. NaOH solution for one hour and wash with distilled water.

It is then kept at room temperature for drying . It takes 2-3 days for Hydrogel to dry. The shape and size of the gel decreases considerably. The rate of drying decreases considerably which can be observed from the loss in weight by the continuous measurement with weighing machine. The gel is further dried in oven at a temperature 40 -45^{0}C. When there is no further loss in weight the Hydrogel is ready for use and testing. The pungent smell of acetic acid also goes off.

Precautions

The thawing of the sample should be at proper temperature i.e less than 30^{0}C. The solution of Chitosan and PVA should be prepared separately and not altogether. There should be proper mixing. The air bubbles should be removed before pouring the solution into petri-dish.

CHAPTER 4

Characterization of Hydrogels

Swelling Study

4.1 Introduction

Hydrogels are polymers characterized by their hydrophilicity, insolubility and responsiveness to swelling in water. They swell to an equilibrium volume and preserve their shape. Hydrophilicity is due to presence of –OH, -COOH , $-SO_3H$ and $–CONH_2$ groups or their salt forms .The stability of shape and insolubility is due to the presence of three dimensional network .The tendency of such networks in dry state to get solvated is referred as high free energy network or hungry networks. Balance between cohesive and dispersive forces in swollen state is responsible for properties of gels. Cohesive forces can be covalent electrostatic, hydrophobic or dipole- dipole interactions. Imbibing large amount of water by Hydrogels results in their poor mechanical properties.

However some natural Hydrogels found in muscles, tendons , cartilage, intestine and blood retain mechanical strength and shape. Hydrogels reinforced with fibers improve the mechanical performance. Polymerization or copolymerization of a variety of monomers with minor amount of cross-linking agents may synthesize Hydrogels. Ionic Hydrogels are prepared combining neutral or ionic monomers or by partial hydrolysis of gel after synthesis . Such gels behave in cyclic manner in solution of different pH. Neutral gels based on water- soluble polymers or copolymer backbones have been prepared and these show Lower Critical Solution Temperature (LCST) phenomenon. If Hydrogel is composed of

lightly cross-linked matrix then gel will shrink significantly over a narrow temperature range.

In general, highly swollen Hydrogels include those of cellulose derivatives, poly(vinyl alcohol), poly(*N*-vinyl-2-pyrolidone) (PNVP) and poly(ethylene glycol) among others. Moderately and poorly swollen Hydrogels are those of poly(hydroxyethyl methacrylate) (PHEMA) and many of its derivatives. In general, a basic hydrophilic monomer can be copolymerized with other more or less hydrophilic monomers to achieve desired swelling properties. Such processes have led to a wide range of swelleable Hydrogels, as Gregonis *et al*. (1976), Peppas (1987, 1997), and others have pointed out.

The knowledge of the swelling characteristics of a polymer is of utmost importance in biomedical and pharmaceutical applications since the equilibrium degree of swelling influences:

(i) The solute diffusion coefficient through these Hydrogels
(ii) The surface properties and surface mobility
(iii) The optical properties, especially in relation to contact lens applications
(iv) The mechanical properties

4.2 Factors Affecting Swelling Behavior Of Hydrogels

Swelling behavior of Hydrogels is mainly affected by the structural and environmental factors. At equilibrium swelling, there is a balance between cohesive and dispersive forces [7.2,7.4]. Factors those affect swelling behavior are given below:

4.3 Structural Factors Affecting Swelling

These are also termed as internal factors and these factors can be tailored by synthetic conditions that determine swell ability of Hydrogels. A Hydrogel of desired swell ability can be designed by proper combination of monomers cross-linking of hydrophilic and hydrophobic moieties and change of monomer concentration results in the formation of Hydrogels of varying swell ability. A porous structure accelerates the extent of swelling. De Boer et al. have reported that solution cross-linked polyethylene could swell to higher limit than bulk cross-linked samples.

Factors increasing swelling	Factors decreasing swelling
Dispersive forces	Cohesive forces
Hydrophilic groups and moieties	Hydrophobic groups and moieties
Low cross-linking density	High cross-linking density
High chain flexibility	Low chain flexibility
High free energy volume	Low free energy volume
Impurities in fluid	Electrostatic repulsion

4.4 Environmental Factors Affecting Swelling

Dynamics of swelling is greatly affected by factors given in the table below:

Physical	Chemical
Temperature	Ph
Ionic strength	Specific ions
Solvents	Chemical agents
Electric field	Biochemical
Mechanical stress	Enzyme substrate
High pressure	Biological agents

4.5 Theory Of Swelling Behavior

The physical behavior of Hydrogels is dependent on their equilibrium and dynamic swelling behavior in water, since upon preparation they must be brought in contact with water to yield the final swollen network structure. Figure given below shows one of two possible processes of swelling. A dry, hydrophilic cross-linked network is placed in water then the macromolecular chains interact with the solvent molecules owing to the relatively good thermodynamic compatibility. Thus the network expands to the solvated state.

Fig: 3(A) Swelling of a network prepared by cross-linking in dry state

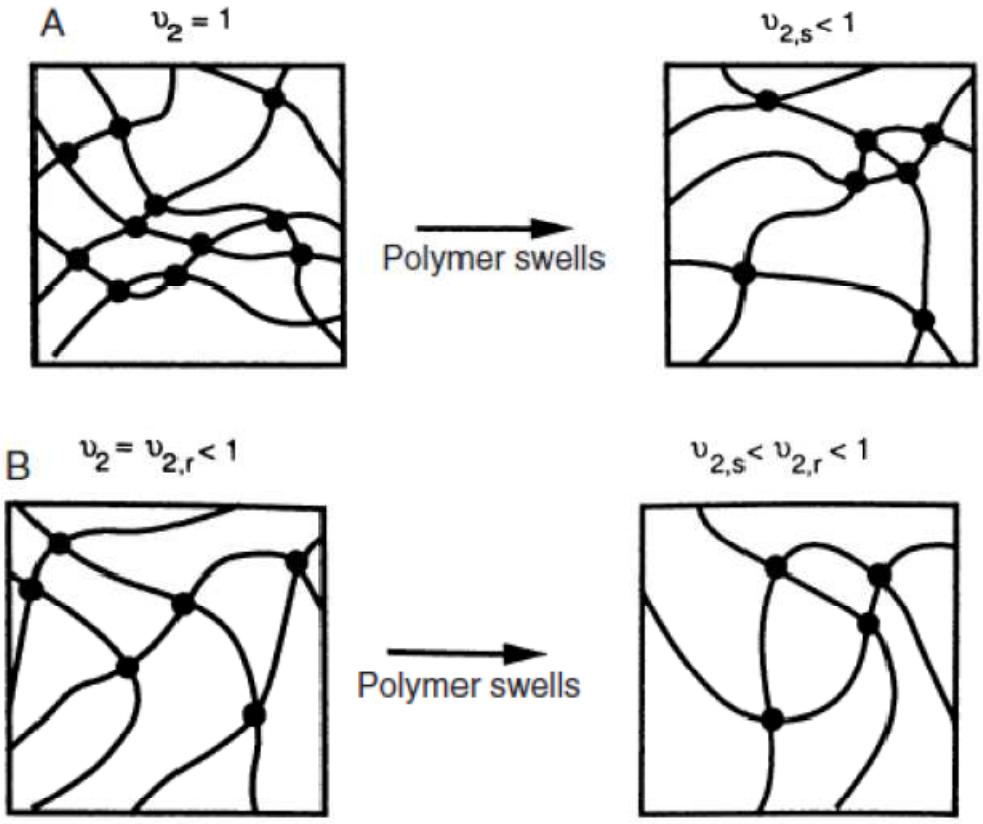


Fig. 3(B) Swelling of a network prepared by cross-linking in solution.

Fig 3

Flory (1953) developed the initial theory of the swelling of cross-linked polymer gels using a Gaussian distribution of the polymer chains. His model describing the equilibrium degree of cross-linked polymers postulated that the degree to which a polymer network swelled was governed by the elastic retractive forces of the polymer chains and the thermodynamic compatibility of the polymer and the solvent molecules.

In terms of the free energy of the system, the total free energy change upon swelling was written as:

$$\Delta G = \Delta G_{\text{elastic}} + \Delta G_{\text{mix}} \qquad (1)$$

Here, $\Delta G_{\text{elastic}}$ is the contribution due to the elastic retractive forces represent the thermodynamic compatibility of the polymer and the swelling agent water. Upon differentiation of Eq. 1 with respect to the water molecules in the system, an expression can be derived for the chemical potential change of water in terms of the elastic and mixing contributions due to swelling.

$$\mu_1 - \mu_{1,0} = \Delta\mu_{\text{elastic}} + \Delta\mu_{\text{mix}} \qquad (2)$$

Here, μ_1 is the chemical potential of water within the gel and $\mu_{1,0}$ is the chemical potential of pure water. At equilibrium, the chemical potentials of water inside and outside of the gel must be equal. Therefore, the elastic and mixing contributions to the chemical potential will balance one another at equilibrium. The chemical potential change upon mixing can be determined from the heat of mixing and the entropy of mixing.

Using the Flory–Huggin's theory, the chemical potential of mixing can be expressed as:

$$\Delta\mu_{mix} = RT\left(\ln(1-2\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1\upsilon_{2,s}^2\right) \qquad (3)$$

where $\chi 1$ is the polymer-water interaction parameter, $\upsilon_{2,s}$ is the polymer volume fraction of the gel, T is absolute temperature and R is the gas constant. This thermodynamic swelling contribution is counterbalanced by the retractive elastic contribution of the cross-linked structure. The latter is usually described by the rubber elasticity theory and its variations (Peppas, 1987). Equilibrium is attained in a particular solvent at a particular temperature when the two forces become equal.

The volume degree of swelling, Q (i.e., the ratio of the actual volume of a sample in the swollen state divided by its volume in the dry state), can then be determined from Eq. 4.

$$\upsilon_{2,s} = \frac{\text{Volume of polymer}}{\text{Volume of swollen gel}} = \frac{V_p}{V_{gel}} = 1/Q \qquad (4)$$

Swelling behavior of various Hydrogels has been explained on the basis of various theory models, especially, to study volume transitions in Hydrogels. Volume transition has been attributed to hydrophobic interactions. Compressible lattice theory has been used to predict transitions by

defining interaction parameters on the basis of large differences in cohesive energy densities of components. It was suggested by hydrogen bonding interactions as well as hydrophobic interactions are crucial for volume transitions. Further dissolution of network chains in the solvent is prevented by cross-links of temporary or of permanent nature. Equilibrium swelling is achieved by balance of osmotic forces of mixing and elastic forces of network.

The weighed amount of Hydrogel is taken and immersed in water at room temperature (around 20^0C) and weighed in an electronic balance after regular interval. The increase in weight of the Hydrogel represents the swelling which is used in the drug release control and waste water treatment. The swollen samples were weighed after the removal of excessive surface water with moistened filter paper.

The swelling ratio (SR) can be calculated from the following equation:

$$\textbf{Swelling Ratio(\%)} = [(W_s - W_d)/W_d] \times 100$$

Where Ws represents the weight in the equilibrium swollen state of the sample at experimental temperature and W_d , the weight of the dry state of the sample.

Equilibrium water content (EWC) of the sample was calculated from the equation:

$$\textbf{EWC (\%)} = [(W_e - W_d)/W_e] \times 100$$

Where W_e is the weight in the equilibrium swollen state of the sample.

4.6 Water Absorption (% Swelling)

The Hydrogels prepared were dipped in distilled water at room temperature(24 - 25^{o}C) and the swelling ratio with time is plotted which is shown in the following diagrams:

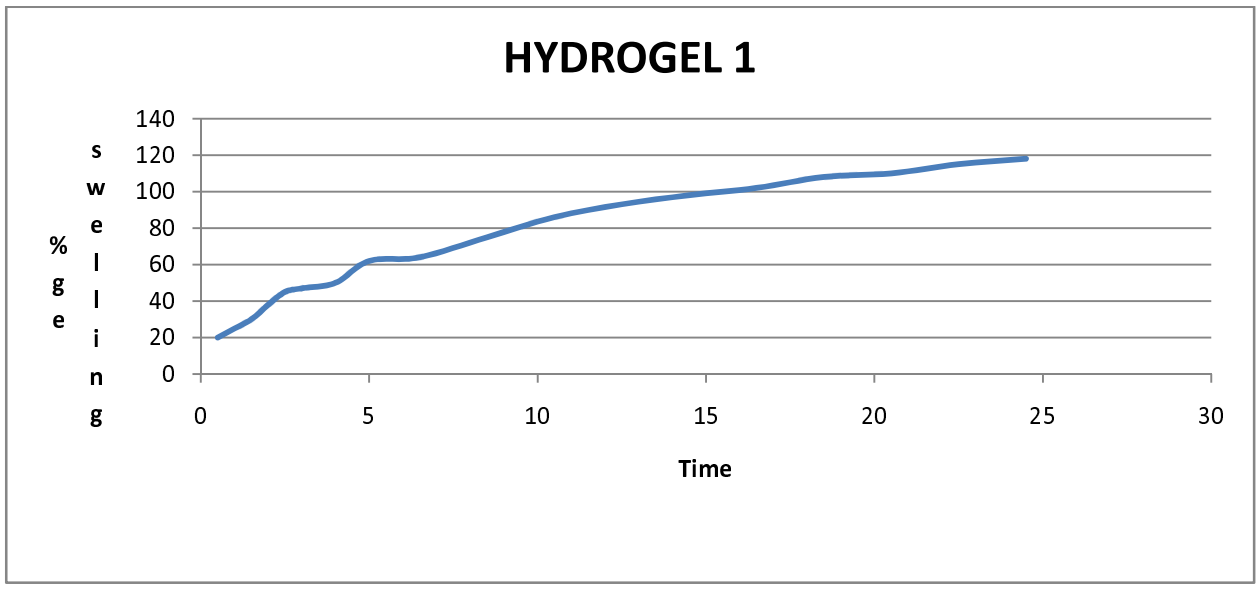


Fig 4 Swelling Behavior of Hydrogel 1 (PVA)

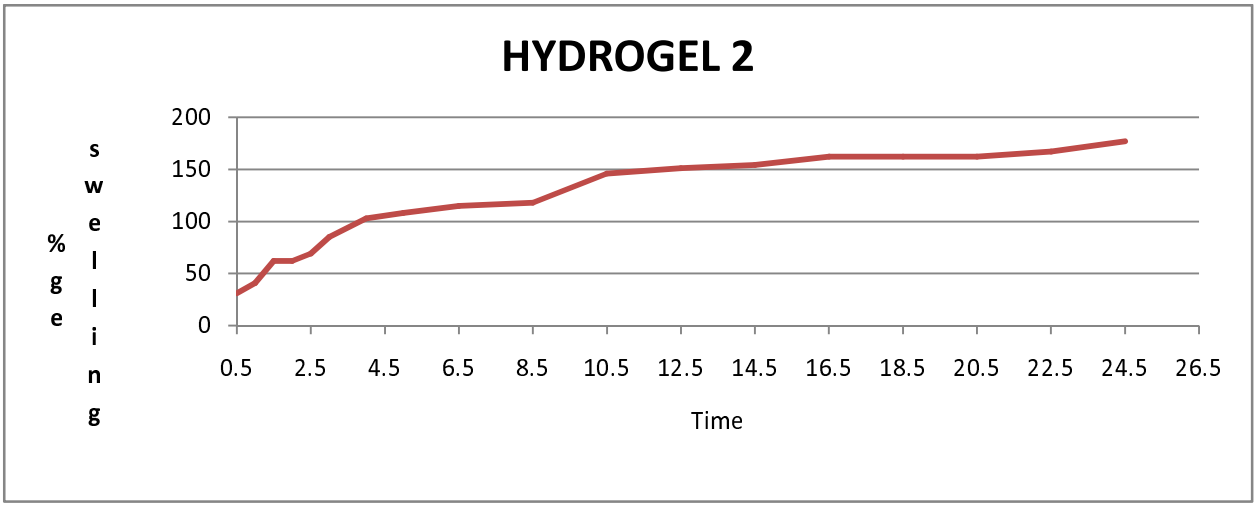


Fig 5 Swelling Behavior of Hydrogel 2 (PVA with 10% Chitosan)

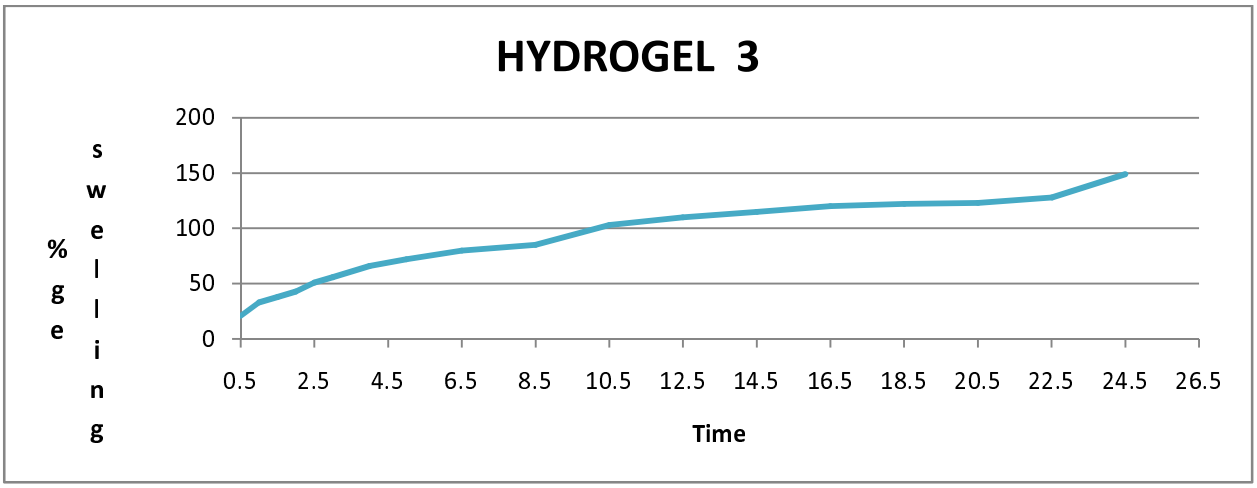


Fig 6 Swelling Behavior of Hydrogel 3 (PVA with 20% Chitosan)

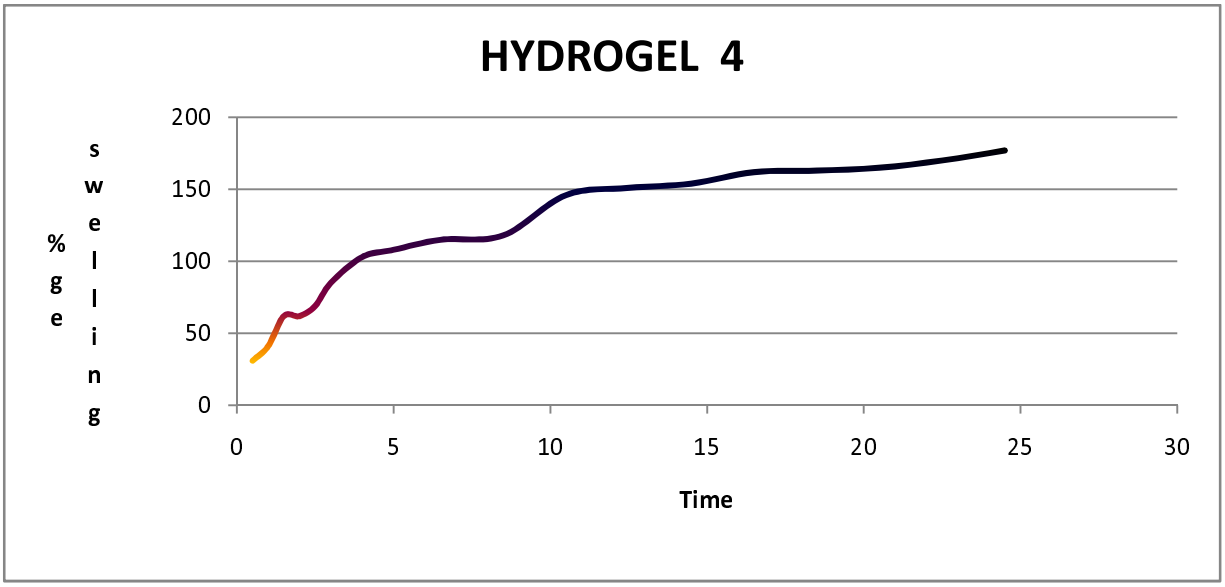


Fig 7 Swelling Behavior of Hydrogel 4 (PVA with 30% Chitosan)

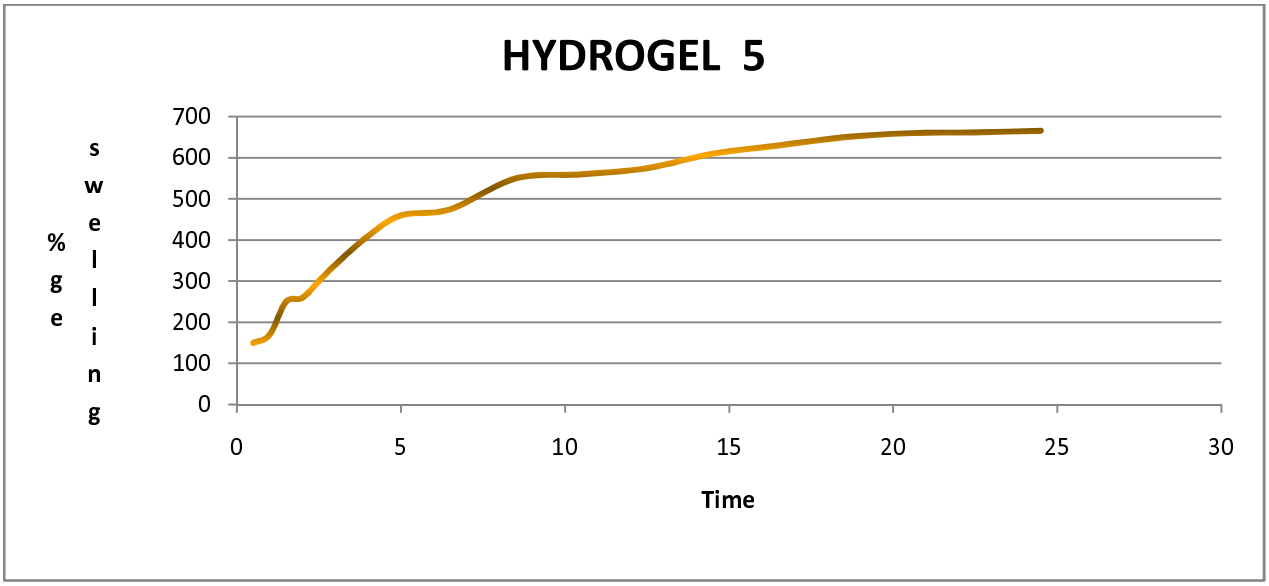


Fig 8 Swelling Behavior of Hydrogel 5 (PVA with 40% Chitosan)

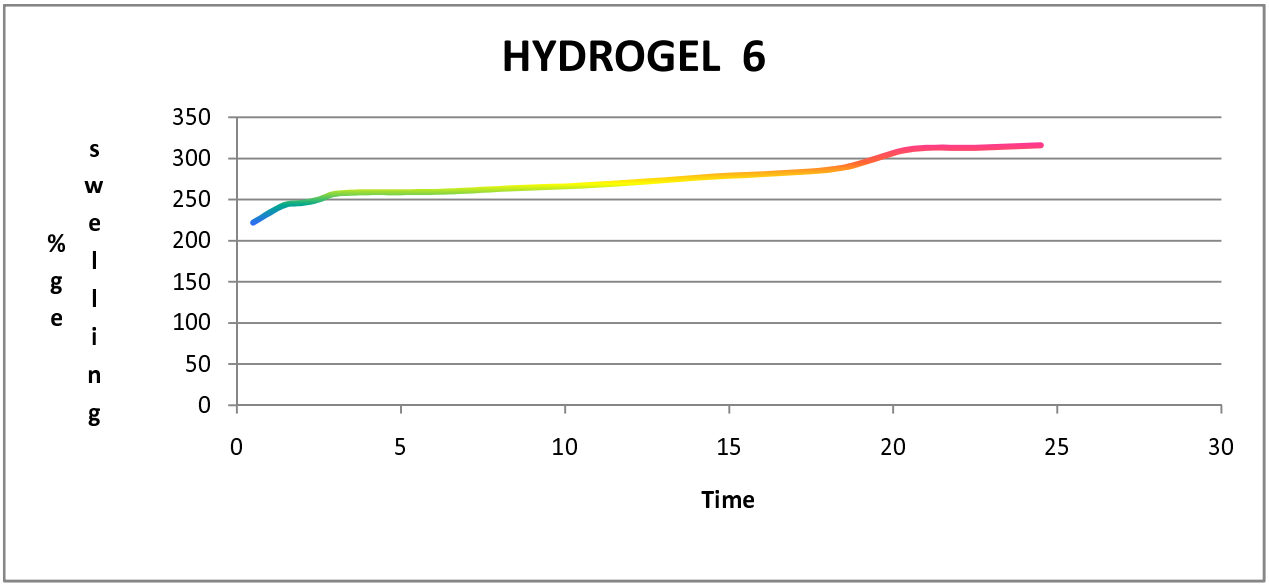


Fig 9 Swelling Behavior of Hydrogel 6 (PVA with 50% Chitosan)

4.7 Fat Absorption Characteristics Of Chitosan

The Chitosan is a different kind of fiber which acts like a powerful magnet for fat and other high calories fat producing substances in the digestive tract. The incredible fat fighting effects of the Chitosan fiber is backed by the extensive scientific research and the Chitosan is proven safe and effective.

One reason why we are fat is because of the lack in nutritional values of the processed food because much of the nutrients are lost during the processing. The other reason for obesity is because of the depleted soils much of our food is already lacking in the essential nutrients.

Empty calories are readily stored as fat which could not be efficiently burned. Fat is burned only when sufficient energy is produced and energy production requires almost every known nutrient.

When the body produces energy it first uses carbohydrates, then protein and then fat. The only fat the body uses, not burns, are essential fatty acids which are unsaturated. They could not be made by the body and must be supplied through the diet.

These essential fats are very important as they are used to help produce harmones and enzymes and act as cofactors to other nutrients. Additionally, they help to lower cholesterol, blood pressure, reduce the risk of heart attacks and are essential for the normal development. Natural forms of the essential fatty acids are found in many vegetables, salmon oil, cod liver oil and fish.

Saturated fats usually come from animal and dairy sources such as cheese, milk, meat, butter and eggs. The body does not use saturated fats in any way except to store them. They clog up our arteries, lead to health disorders and when we see them on our hips, lips, thighs, stomach and under our chins etc.

There is an old saying about the fat ,"If it's past the lips it's on the hips. "

Chitosan has a natural powerful magnetic attraction for lipids, fats, and bile in the digestive tract and actually binds with them preventing them from being absorbed into the blood stream.

Chitin is structurally similar to cellulose which is a plant fiber except in Chitosan, acetylamino groups are in place of hydroxyl groups in its molecular composition.

Chitosan is derived from chitin by removing and refining the acetyls through a process called de-acetylation. Removing acetyls groups results in an unstable amino-polysaccharide molecule with strong positive polarity. Chitosan's positive polarity attracts negatively charged molecules and ionic ally bonds them to the Chitosan.

Within the digestive system the Chitosan dissolves and forms a positively charged gel. Negatively charged molecules of the fats, lipids and bile attach strongly to the Chitosan sites from where the acetyl groups were removed. This electrolytic bonding causes large polymer compounds to be formed that can not be broken down by the digestive process.

The Chitosan acts as a coagulating agent for other activated solids, bulk wastes and fibers trapping them in the polymer. These solids can

contain high calorie molecules such as complex sugar chains and high calorie carbohydrate micelles. These are substances that often get converted into fat which is stored in the body. As the Chitosan polymer grows, it becomes too large to be absorbed through the lining of the digestive tract. Eventually the polymer is excreted as waste from the digestive system, carrying away the attached fat and other potential fat producing substances.

In the case of the unsaturated fatty micelles, they are not burnt for energy but stored as fat. Chitosan prevents the absorption of these fat producing micelles. Excessive bile acids are believed to contribute to colon and prostate cancer, so their elimination may be another positive feature of Chitosan.

Chitosan is also called lipophilic, meaning that it is chemically attracted to or loves fat. Generally fibers are hydrophilic which means they repel fat and attract water. Chitosan actually captures and inactivates lipids, fats and cholesterol. Research shows that Chitosan can bind significantly higher amounts of fats than other fibers can entrap.

It can be summarized that the Chitosan offers everyone the potential of achieving permanent weight loss and other wonderful health benefits. Chitosan can be viewed as a miracle worker. So Chitosan can be used as bulk to the digestive system and for colon cleansing.

4.8 Fat Absorption Studies Of Prepared Hydrogels

For studying the fat absorption the weighed amount of sample is placed in butter oil for four hours at a temperature of 37-39^{0}C. The percentage of

fat absorbed can be calculated by calculation of increase in the weight. The following graph is obtained for the synthesized Hydrogels:

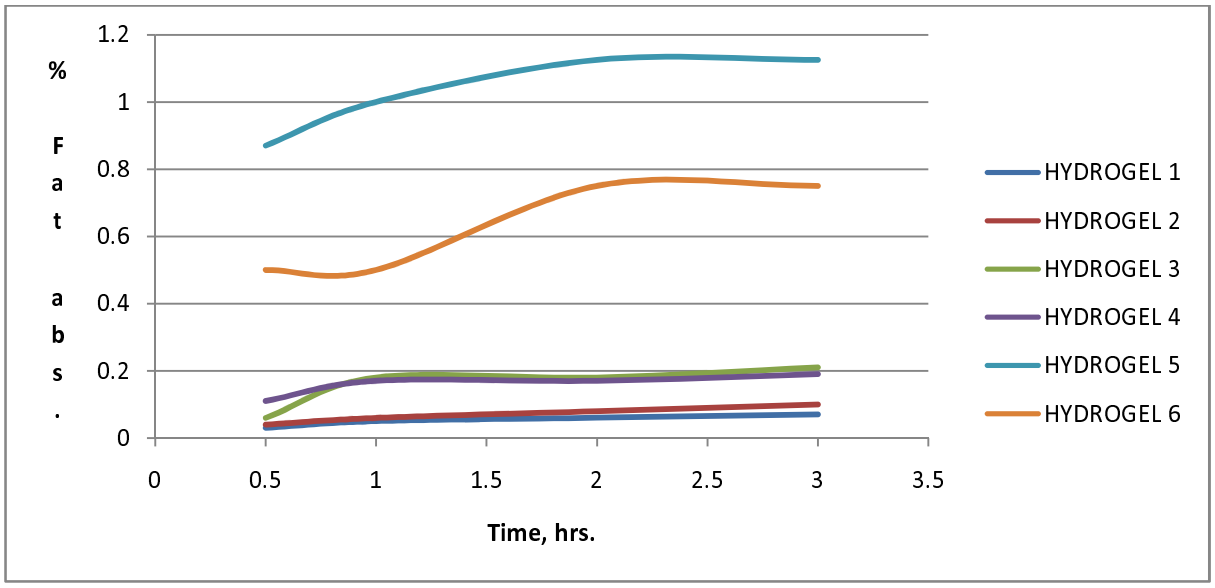


Fig 10 Fat Absorption Behaviour Vs. Time Of Hydrogels

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4.9 Swelling Of Hydrogels With Temperature

The Hydrogels were placed in distilled water for two hours at temperature 30, 40 and 50^0 C and the following graphs are obtained:

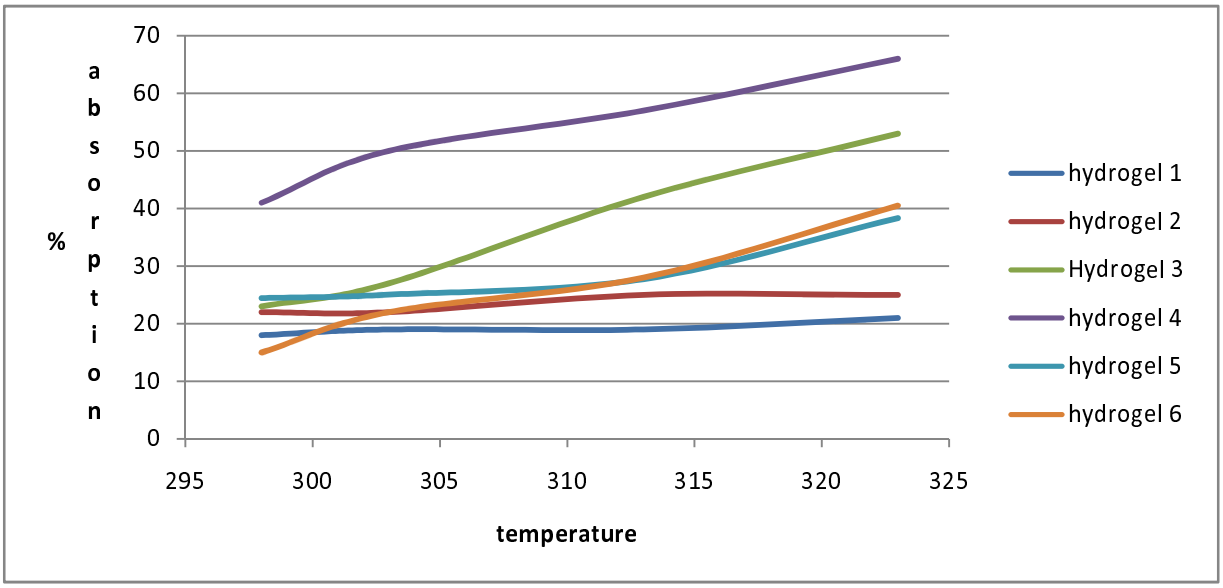


Fig 11 Swelling Behavior Of Hydrogels With Temperature

4.10 De-Swelling Studies Of Hydrogels

It is important to evaluate how long the polymer matrix can hold water molecules within the matrix. This property is quite important and mostly depends on the chemical composition and structure of the Hydrogels. The Hydrogels are first placed in distilled water for 36 hours so that equilibrium water amount is absorbed. The Hydrogels are then removed and put in petri dish in open air. The percent loss in weight with time is recorded. In the present study, therefore, the progress of the de-swelling process is monitored for the prepared Hydrogels. The different de-swelling graphs of Hydrogels have been given below:

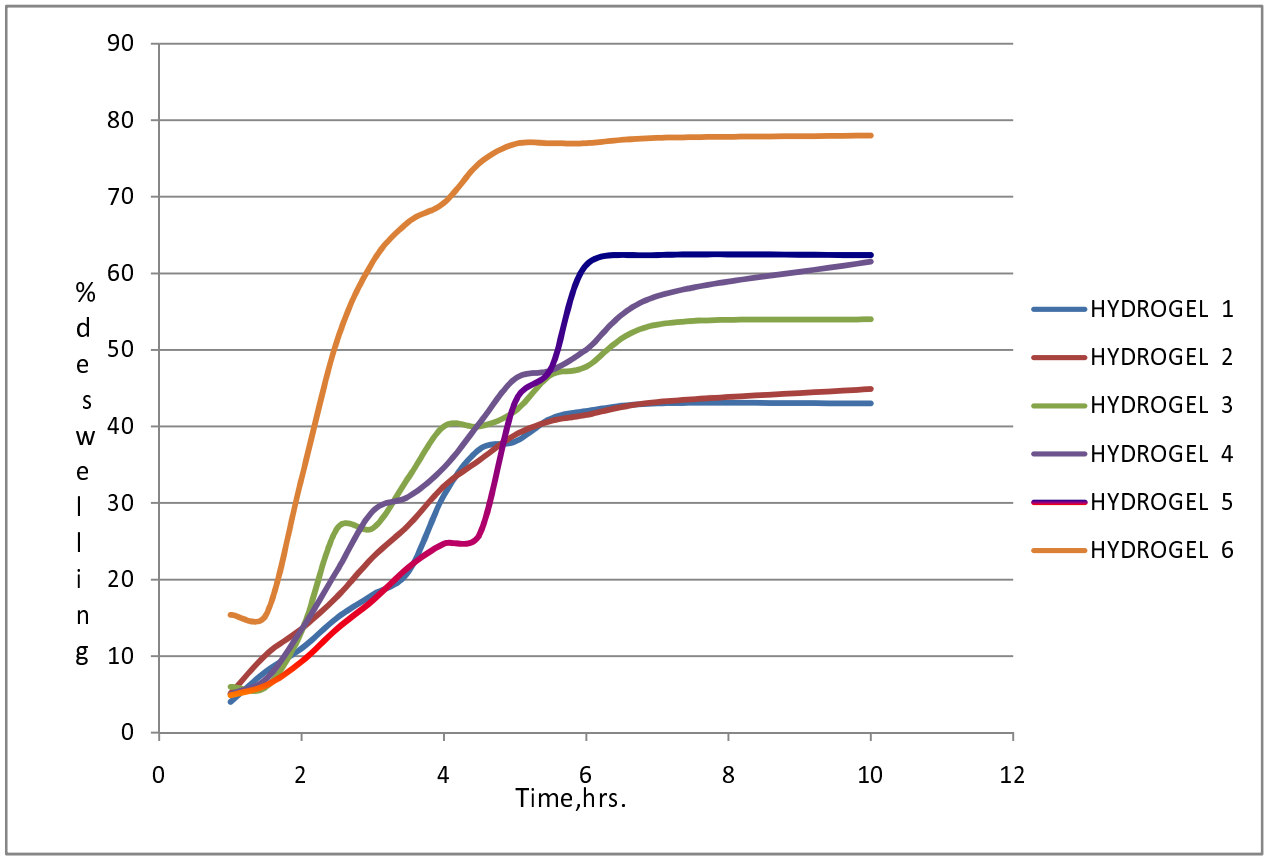


Fig 12 De-swelling Behavior Of Hydrogels With Time

4.11 Swelling Of Hydrogels In Different pH Medium

The Hydrogels were placed in medium of different pH of 5,6,7,8 and 9 for two hours and the percentage swelling is calculated. The following graphs are obtained for different Hydrogels:

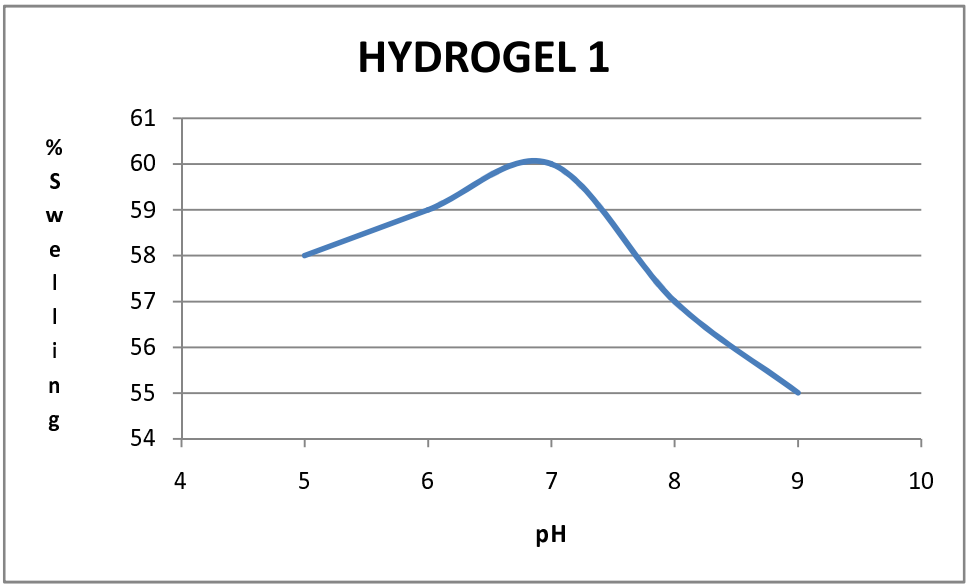


Fig 13 Swelling Behavior With pH Of Hydrogel 1 -PVA

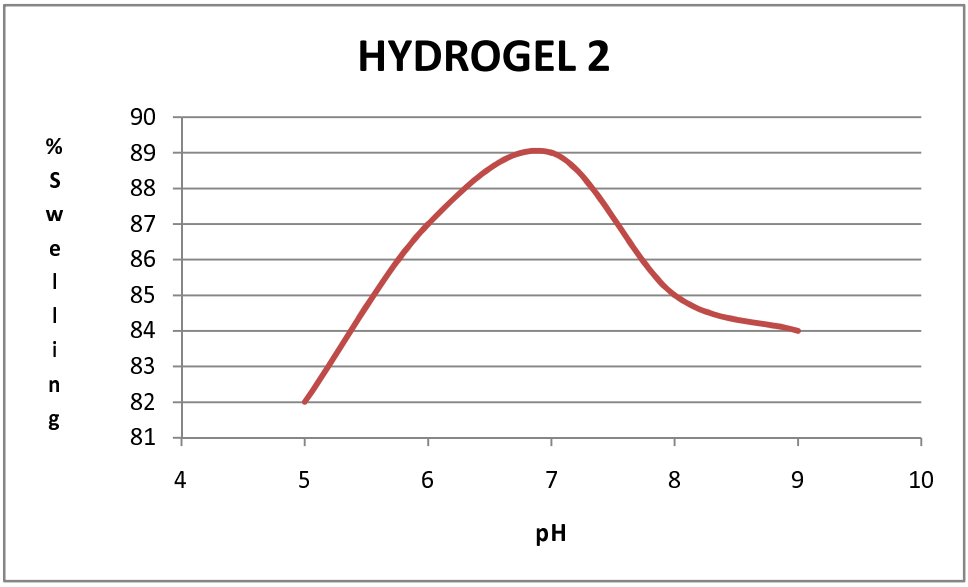


Fig 14 Swelling Behavior With pH Of Hydrogel 2 -PVA With 10% Chitosan

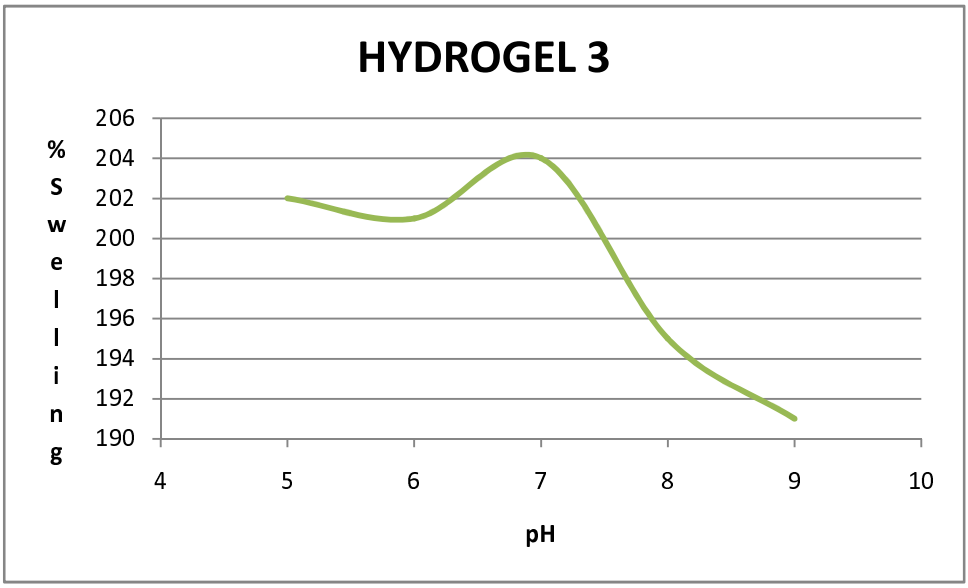


Fig 15 Swelling Behavior With pH Of Hydrogel 3

-PVA With 20% Chitosan

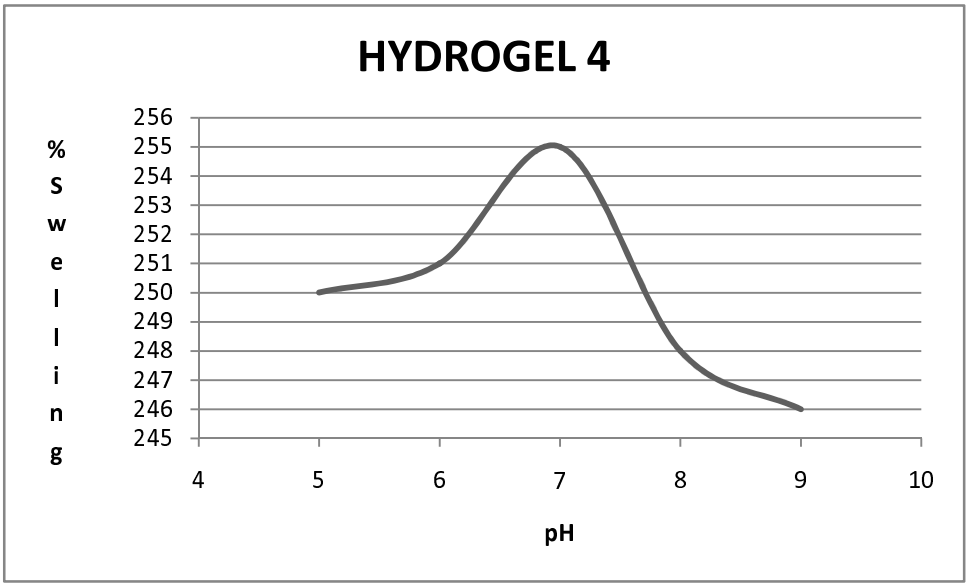


Fig 16 Swelling Behavior With pH Of Hydrogel 4

-PVA With 30% Chitosan

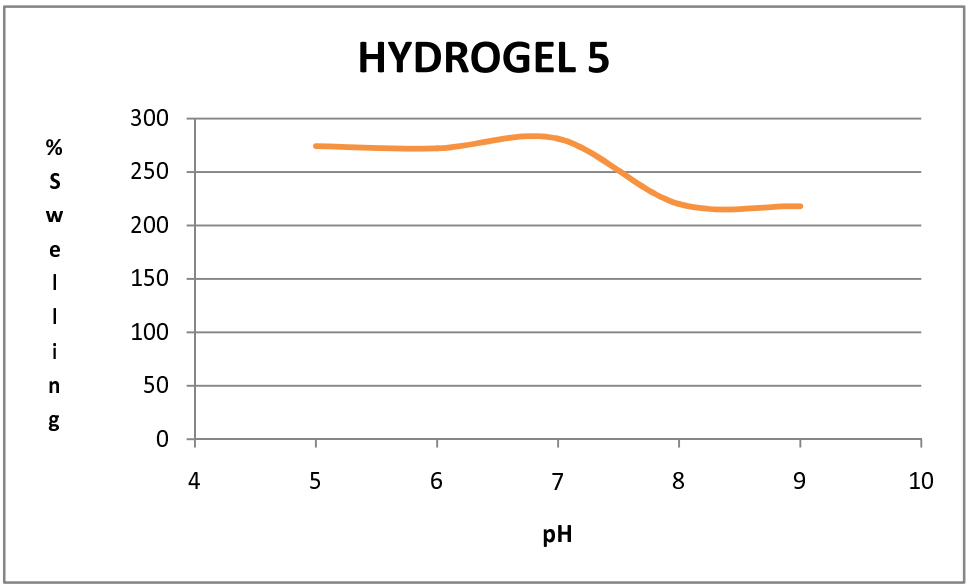


Fig 17 Swelling Behavior With pH Of Hydrogel 5 -PVA With 40% Chitosan

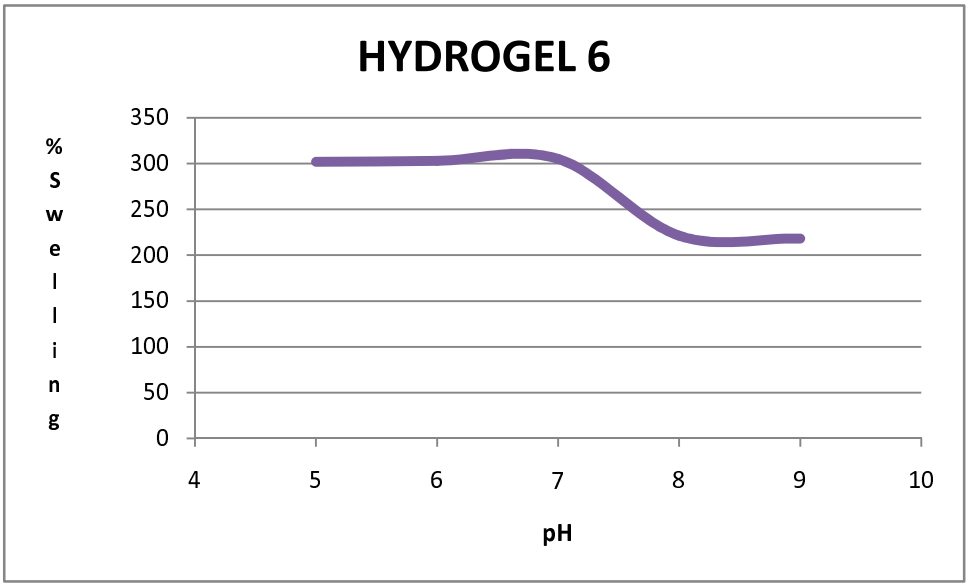


Fig 18 Swelling Behavior With pH Of Hydrogel 6 -PVA With 50% Chitosan

4.12 Fourier Transform Infrared Spectroscopy (FTIR) of Hydrogels

This spectroscopy deals with infrared region of the electromagnetic spectrum. It covers a range of techniques mostly based on absorption spectroscopy. It is used to identify and study chemicals. A common laboratory instrument used for this technique is a Fourier Transform Infrared Spectrometer (FTIR).

The infrared portion of the electromagnetic spectrum consists of the near, mid and far infrared. The near-IR approximately 14000-4000 cm^{-1}(0.8-2.5 micro meter wavelength) can excite overtone or harmonic vibrations. The mid infrared approximately 4000-400 cm^{-1}(2.5-25micrometer) may be used to study the fundamental rotational-vibrational structure. The far infrared approximately 400-10 cm^{-1}(25-1000 micrometer) lying adjacent to the microwave region has low energy and may be used for rotational spectroscopy.

Fig 19- ***FTIR Spectrometer***

A FTIR spectrometer simultaneously collects spectral data in a wide spectral range while dispersive spectrometer measures intensity over a narrow range of wavelength at a time. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical algorithm)is required to convert the raw data into the actual spectrum. The first low cost spectrophotometer capable of recording an infrared spectrum was produced in 1957. The FTIR of prepared Hydrogels was performed. The following spectra are obtained :

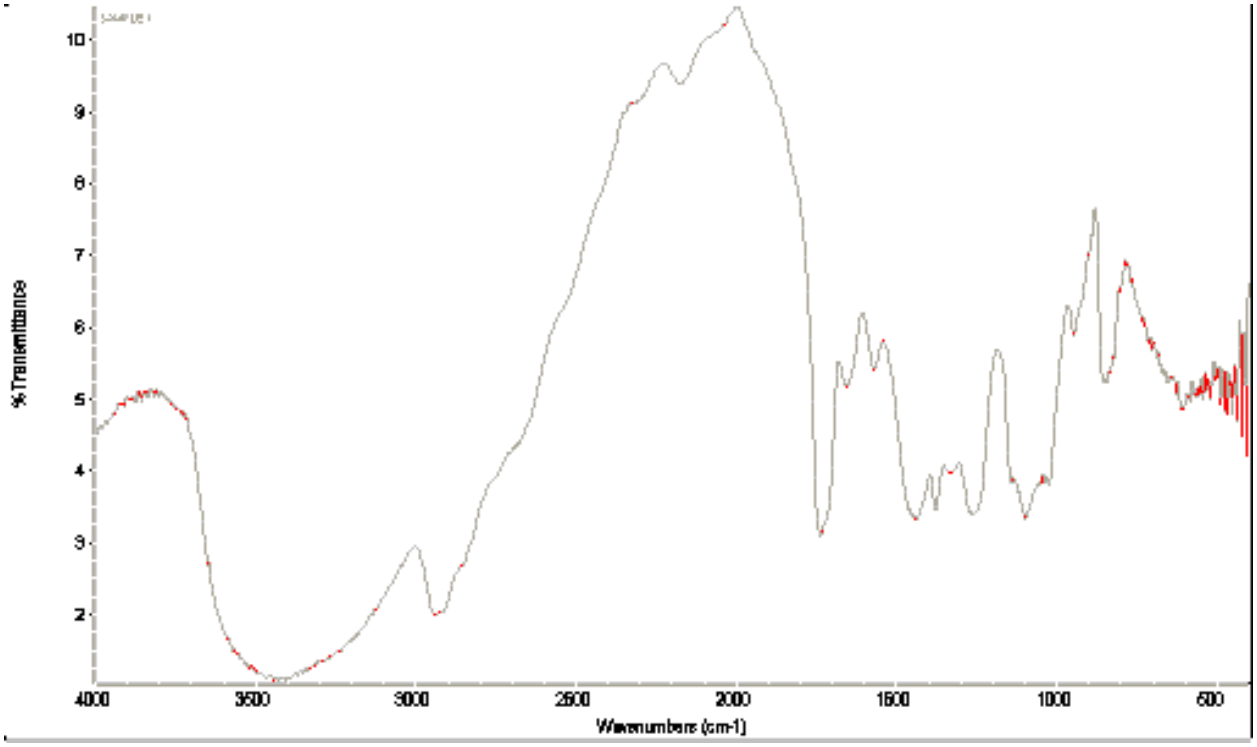


Fig 20 FTIR Spectrum Of PVA

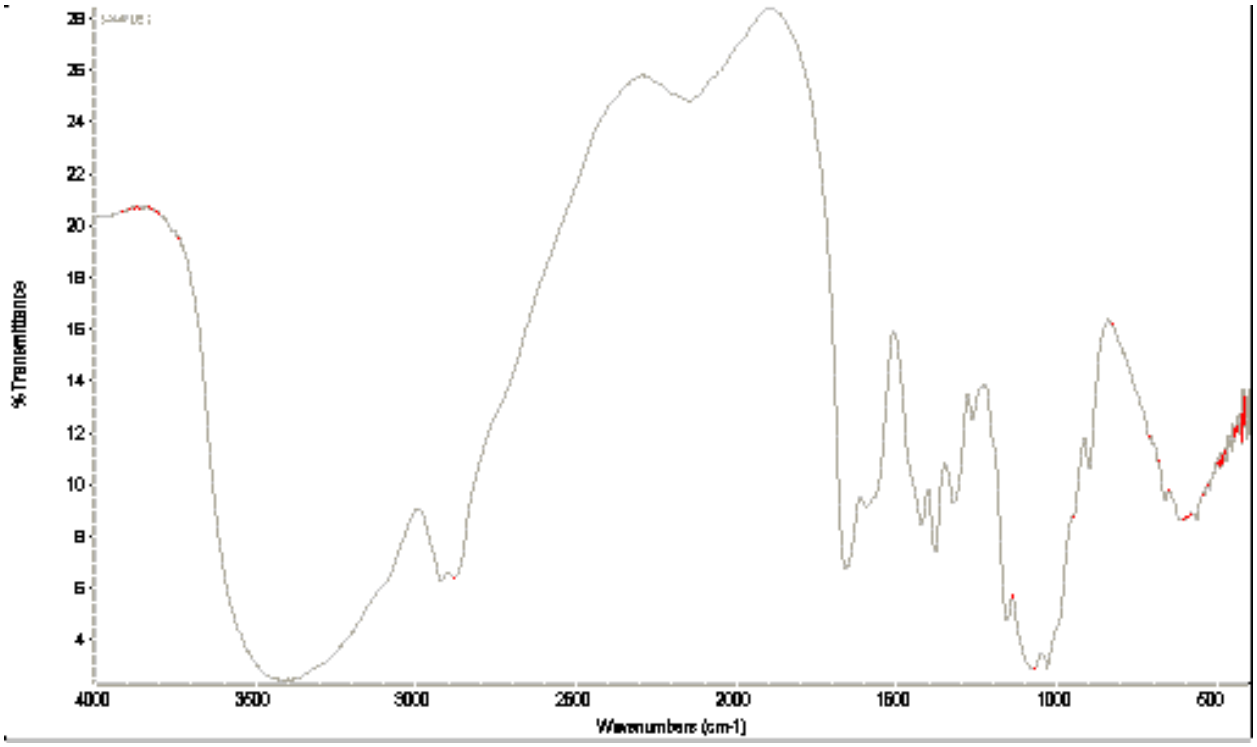


Fig 21 FTIR Spectrum Of Chitosan

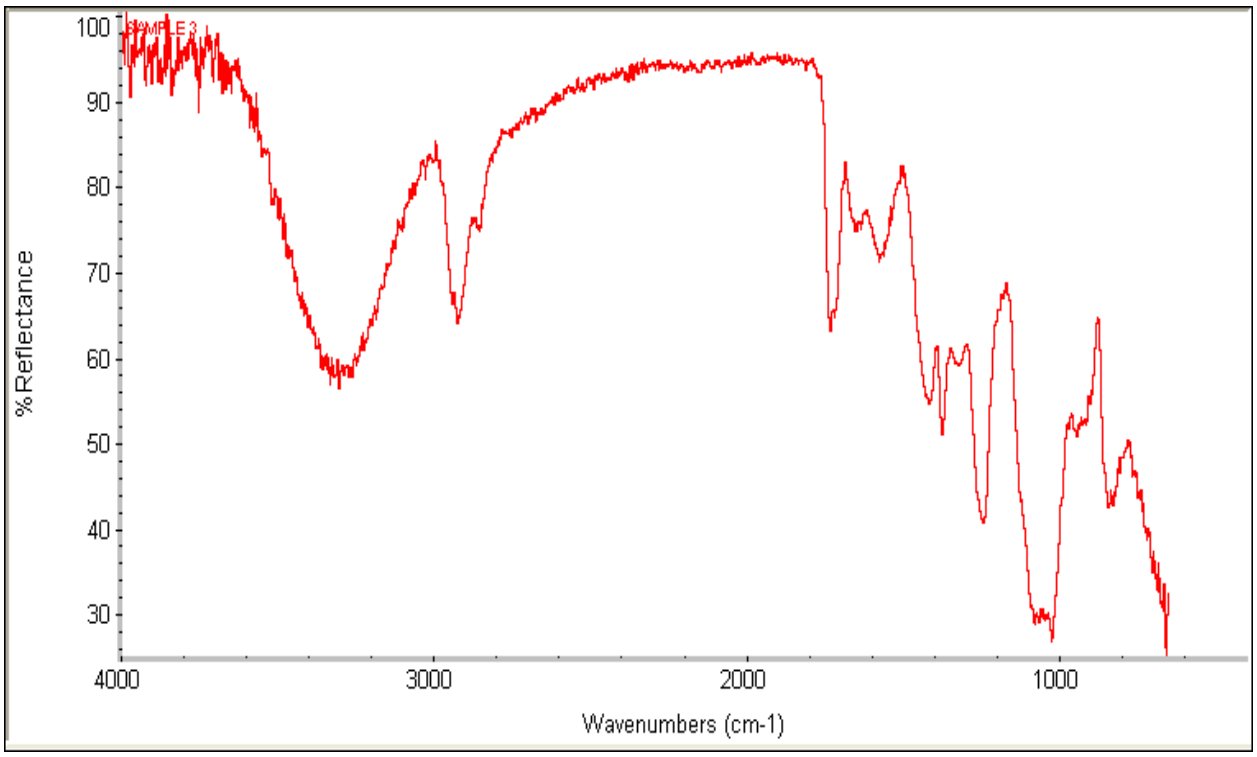


Fig 22 FTIR Spectrum Of Hydrogel 1 (PVA With 10% Chitosan)

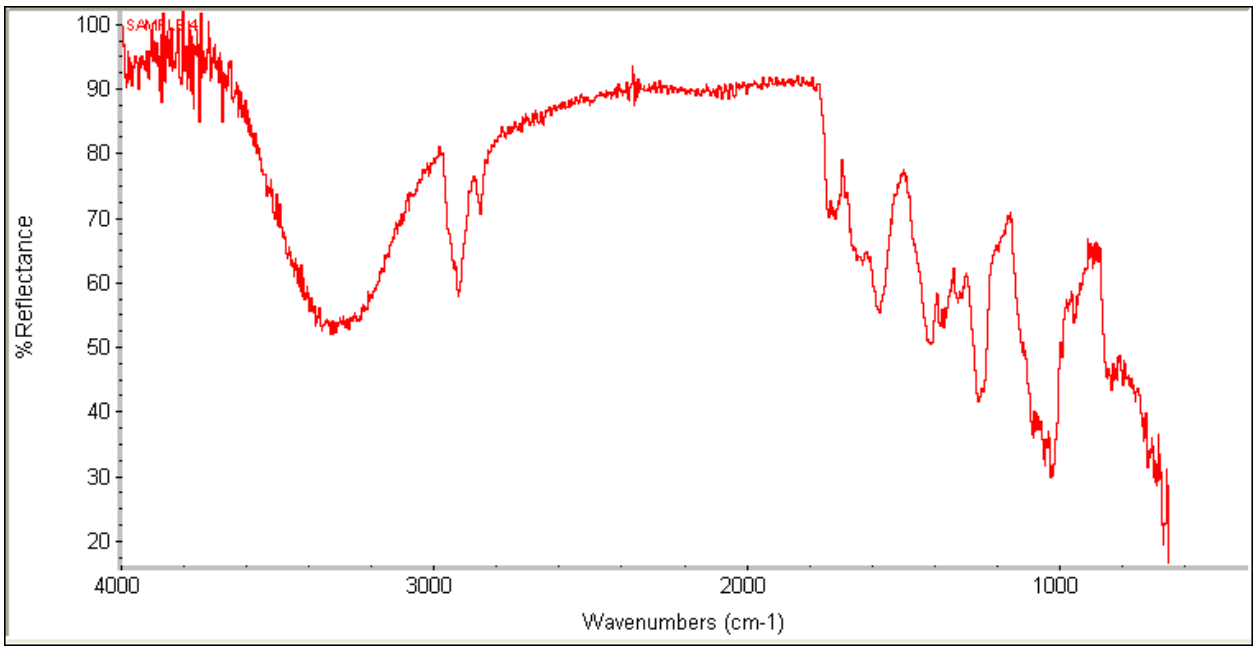


Fig 23 FTIR Spectrum Of Hydrogel 2 (PVA With 20% Chitosan)

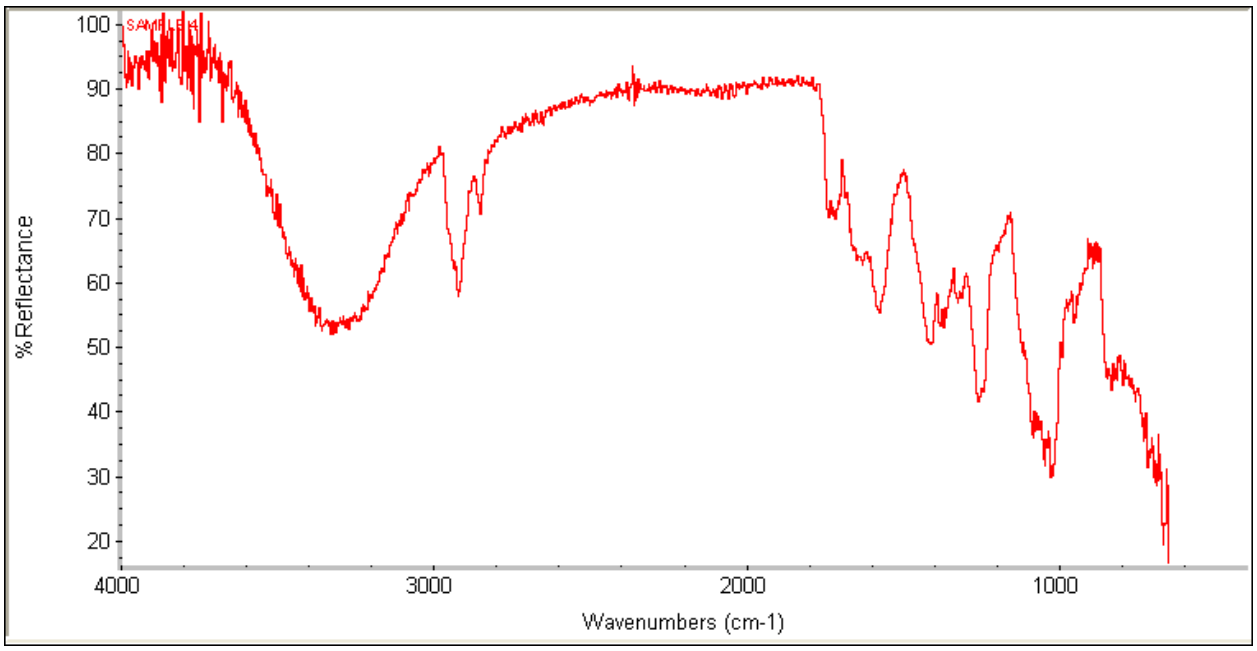


Fig 24 FTIR Spectrum Of Hydrogel 3 (PVA With 30% Chitosan)

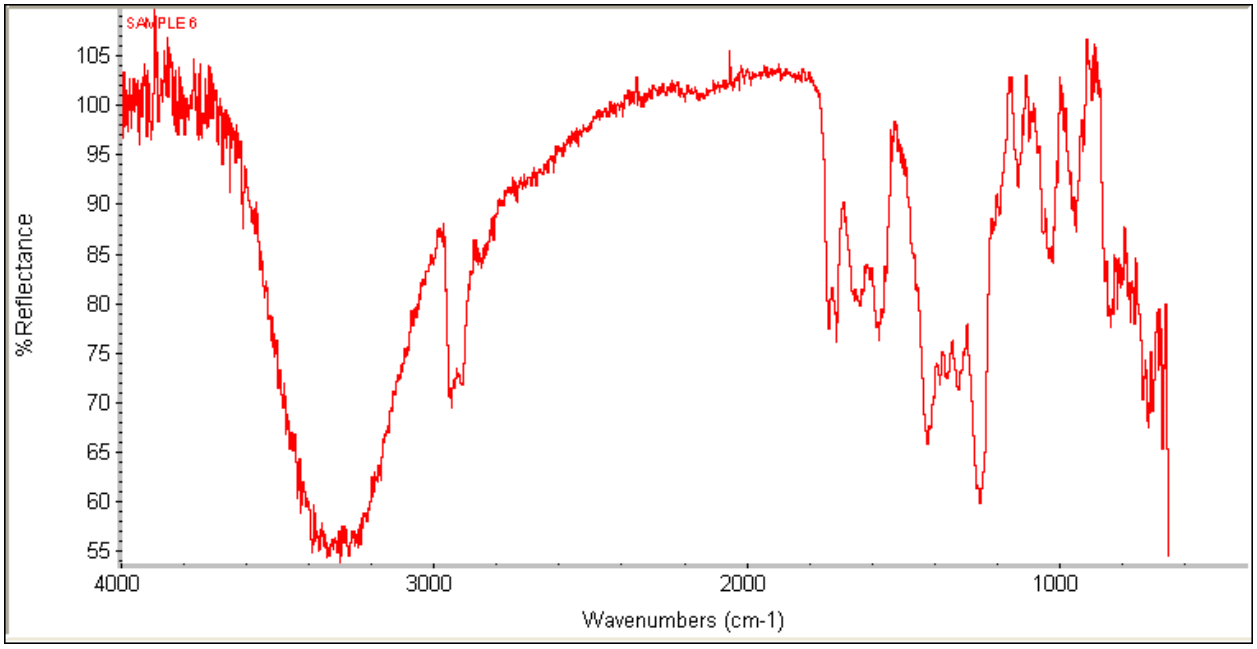


Fig 25 FTIR Spectrum Of Hydrogel 4 (PVA With 40% Chitosan)

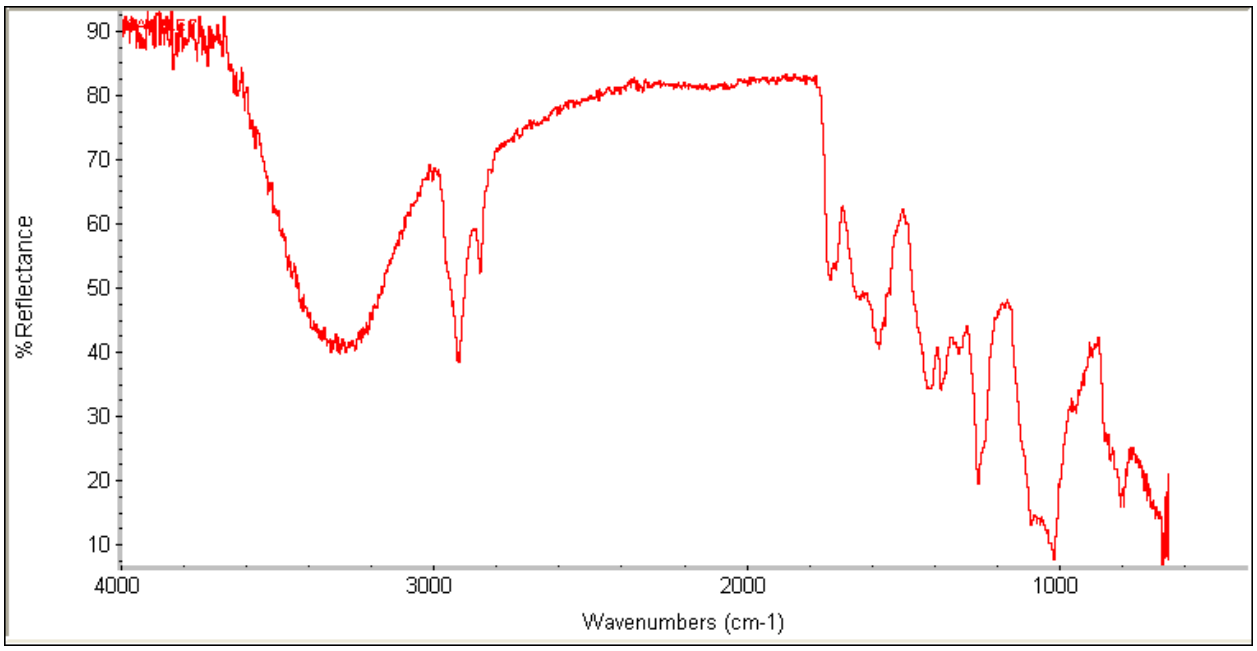
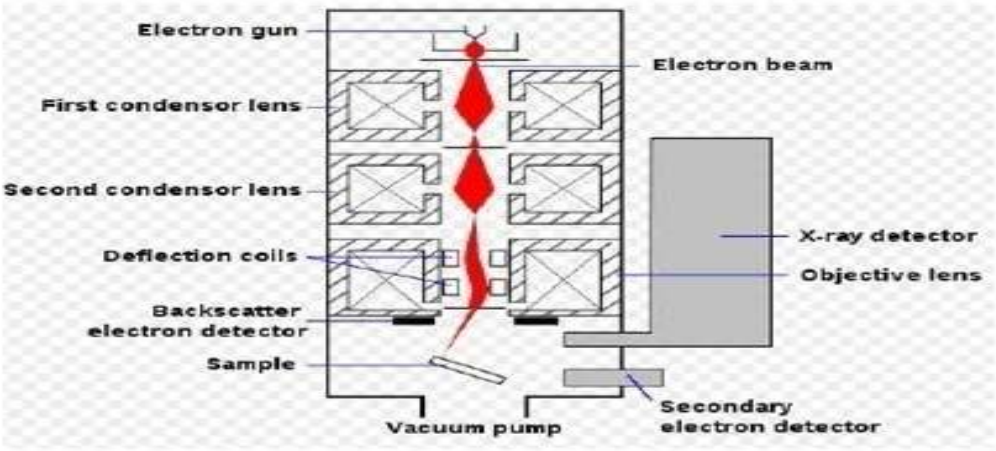


Fig 26 FTIR Spectrum Of Hydrogel 5 (PVA With 50% Chitosan)

4.13 Scanning Electron Microscopy (SEM) Of Hydrogels

A scanning electron microscope is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern.

SEM opened sample chamber



Schematic diagram of SEM

Fig 27 Scanning Electron Microscope

The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography,

composition and other properties such as electrical conductivity. The first SEM image was obtained by Max Knoll, who in 1935 obtained an image of silicon steel showing electron channeling contrast. The types of signals produced by a SEM include secondary electrons, back-scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence), specimen current and transmitted electrons. The signals result from interactions of the electron beam with atoms at or near the surface of the sample. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample causing a higher energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.

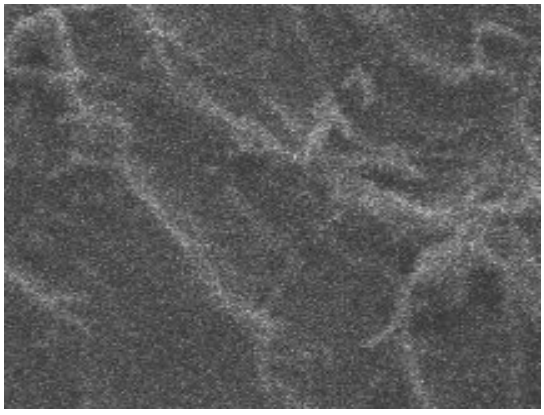


Fig 28 SEM Image of PVA

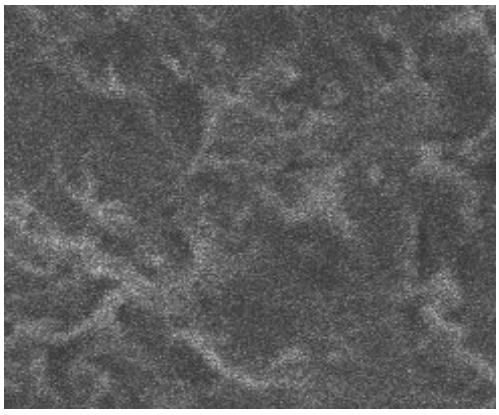


Fig29 SEM Image of PVA With 10% Chitosan

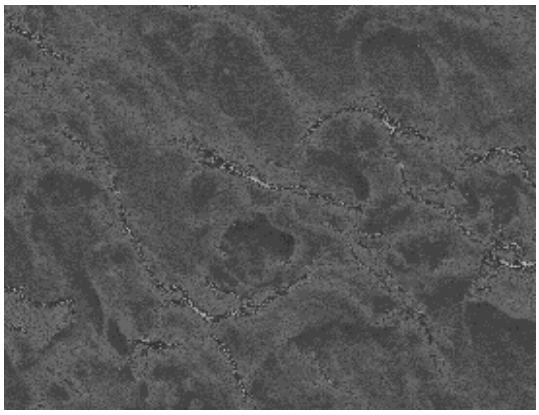


Fig 30 SEM Image Of PVA With 20% Chitosan

Fig31 SEM Image Of PVA With 30% Chitosan

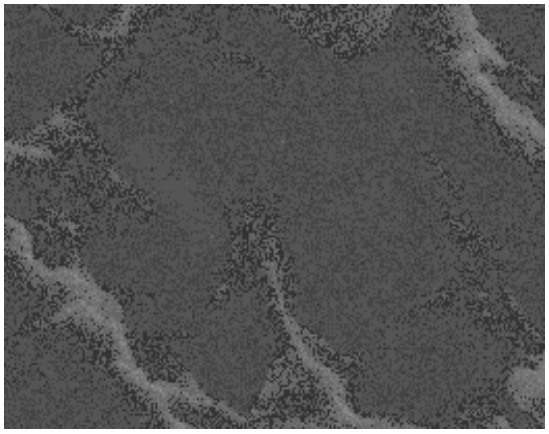


Fig 32 SEM Image Of PVA With 40% Chitosan

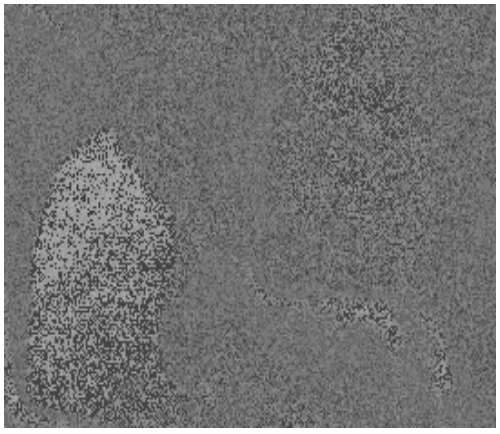


Fig 33 SEM Image Of PVA With 50% Chitosan

In a typical SEM, an electron beam is thermo-ionically emitted from an electron gun fitted with a tungsten filament cathode. Other types of electron emitters include lanthanum hexaboride (LaB6) cathodes. The electron

beam, which typically has an energy ranging from 0.5 keV to 40 keV, is focused by one or two condenser lenses to a spot about 0.4 nm to 5 nm in diameter. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron column typically in the final lens which deflect the beam in the *x* and *y* axes so that it scans in a raster fashion over a rectangular area of the sample surface. When the primary electron beam interacts with the sample, the electrons lose energy by repeated random scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume which extends from less than 100 nm to around 5 μm into the surface. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering. The beam current absorbed by the specimen can also be detected and used to create images of the distribution of specimen current.

Electronic amplifiers of various types are used to amplify the signals which are displayed as variations in brightness on a cathode ray tube. The raster scanning of the CRT display is synchronized with that of the beam on the specimen in the microscope and the resulting image is therefore a distribution map of the intensity of the signal being emitted from the scanned area of the specimen. The image is digitally captured and displayed on a computer monitor and saved to a computer's hard disk. The above pictures show the SEM images of the prepared Hydrogels.

4.14 X-Ray Diffraction of Hydrogels:

X-radiation (composed of X-rays) is a form of electromagnetic radiation. X-rays have a wavelength in the range of 0.01 to 10 nanometers. X- ray machines were introduced in 1974 by Samuel Eilenberg. X-ray powder diffraction is a non-destructive technique widely applied for the characterization of crystalline materials. The method has been traditionally used for phase identification, quantitative analysis and the determination of structure imperfections. Now application has been extended to new areas, such as the determination of crystal structures and the extraction of three-dimensional micro-structural properties. Various kinds of micro and nano-crystalline materials can be characterized from X-ray powder diffraction, including in-organics, organics, drugs, minerals, zeolites, catalysts, metals and ceramics.

The observed diffraction line profiles in a powder diffraction pattern are distributions of intensities I(2